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Drug Master File

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Name of Applicant

The Department of Transfusion Medicine
W. G. Magnuson Clinical Center
National Institutes of Health
10 Center Dr.
Bld. 10 Rm 1C 711 MSC 1184
Bethesda, MD 20892-1184

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Facility, Operational and Quality Systems
for Manufacture of Cellular Therapy Products

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Cell Processing Section
Department of Transfusion Medicine
NIH Clinical Center

Master File of Facility, Operational and Quality Systems for Manufacture of Cellular Therapy Products

Cell Processing Section
Department of Transfusion Medicine
Warren Grant Magnuson Clinical Center
National Institutes of Health
Bethesda, MD 20892

Table of Contents

List of Attachments

1. Organizational Chart

2. Relationship between Institute investigators and CC regarding protocol design, implementation, and quality assurance

3. Quality control schedule

4. CPS facility floorplan

5. Personnel flow within CPS facility

6. Product and materials flow within CPS facility

7. Waste flow within CPS facility

8. Air pressure differentials and flow within CPS facility

9. Donor selection & eligibility tables:

    9-A Living autologous donors
    9-B Living allogeneic family-related donors for products other than cord blood
    9-C Cord blood donors (autologous or allogeneic family-related)
    9-D Cadaveric donors

10. Cell separation and selection procedures

11. Assay/testing tables:

    11-A Donor transmissible disease testing
    11-B Sterility testing (bacterial and fungal culture)
    11-C Mycoplasma PCR testing
    11-D Mycoplasma culture
    11-E Endotoxin testing
    11-F Automated cell counting
    11-G Trypan blue viability testing
    11-H Flow cytometric 7-AAD viability testing
    11- I Flow cytometric phenotyping of cells
    11-J Hematopoietic colony assays
    11-K Testing for ABO group, Rh type, and unexpected RBC antibodies
    11-L HLA antibody screening

I. Introduction

A. Purpose of Master File

The purpose of this Master File is to describe the critical procedures, policies, and systems related to the facility, operations, and quality management of the Cell Processing Section (CPS), Department of Transfusion Medicine (DTM) at the National Institutes of Health (NIH) Clinical Center (CC). The information contained in this Master File will serve as a summary that can be cross-referenced by NIH investigators and other parties submitting Investigational New Drug (IND) applications that involve products manufactured by CPS.

B. CPS Mission

The central mission of CPS is to provide services to the NIH Institutes to support their intramural clinical trials. These services include (1) performing research aimed at development, evaluation, and validation of new manufacturing processes for cellular therapies and (2) manufacture of cellular therapy products for approved clinical trials. The overwhelming majority of these trials are early phase (I/II) trials not intended to result in development of a commercial product. To provide these services, CPS operates a core facility for the manufacture, storage, and distribution of cellular therapy products.

C. CPS Organizational Relationships

An organizational chart is provided in **Attachment 1**. CPS is one of four sections of the DTM, which provides a wide range of blood, blood transfusion, and cellular therapy services for both patient care and research support at the NIH Clinical Center. CPS is administered by, and funded through, the NIH Clinical Center, and not directly by the individual Institutes. CPS is subject to guidelines and policies of the Department of Health and Human Services (DHHS), the Public Health Service (PHS), the NIH, the Clinical Center, and the Department of Transfusion Medicine. All quality-related activities of CPS are guided by the quality programs of the NIH Clinical Center and the DTM; these quality programs, which are aligned with each other, are described in Section II (Quality Program).

D. Role of CPS in Clinical Trials

The Institutes of the NIH administer clinical trials involving a wide range of cellular therapies, many of which are supported by CPS. A given clinical trial may involve the manufacture of one or more cellular therapy products, which may be IND-related or not.

The relationship and interactions between the CPS/DTM and the NIH Institute investigators with regard to Good Clinical Practice (GCP) requirements for protocol development, product manufacturing issues and protocol implementation and quality assurance, including reporting of adverse events, are shown in **Attachment 2**.

Senior physician-investigators employed by the NIH Institutes serve as Principal Investigators (PIs) for clinical protocols and their associated IND applications, if required. Physicians and sometimes other staff members of the DTM are designated as Associate Investigators on most clinical protocols and on IND applications. PIs interact extensively with the CPS Medical Director and other CPS staff during all phases of protocol development and implementation.

PIs are responsible for submission of the clinical protocol to the appropriate scientific review panel, Institutional Review Boards (IRBs) and other applicable review panels. They are also responsible for overall conduct of the clinical trials. For all Institutes except the National Cancer Institute (NCI), the protocol PI or a Responsible Investigator (RI) serves as the holder of the IND when one is required but not held by a commercial sponsor. For NCI protocols, INDs not held by commercial sponsors are held by either the protocol PI/RI or by NCI's Cancer Therapy Evaluation Program (CTEP).

E. Products Manufactured and Handled by CPS

A list of products manufactured, handled, or stored by CPS, by calendar year, will be provided as an attachment to the Master File transmittal letter, and in annual updates. This listing includes IND-related clinical products, non-IND-related clinical products, and non-clinical products manufactured for research or validation studies.

II. Quality Program

A. General

The DTM, and CPS—as one of its sections—have established an organizational commitment to quality management and have a well-defined quality program. The CPS quality program is fully integrated with that of DTM. The DTM Quality Plan is described in DTM-SOP-0006 (Department of Transfusion Medicine Quality Plan).

B. Program Organization, Authority and Responsibility

DTM personnel responsible for quality management are listed in **Attachment 1**. In addition, responsibilities of specific managerial positions with regard to the quality program are listed below. The DTM Quality Systems Officer is responsible for ensuring that the quality program is established and maintained and reports to management on the performance of the quality program, but does not perform manufacturing functions in the CPS.

- The DTM Department Chief has ultimate responsibility for ensuring the quality of the products and services provided by the DTM, exercises control of the department in all matters relating to compliance with regulatory and accrediting agency guidelines, and directs organizational planning and management efforts related to quality.

- The DTM Quality Systems (QS) Officer coordinates, monitors and facilitates all quality assurance activities in the department, ensures that quality of products manufactured by DTM meets applicable regulatory and accreditation requirements, ensures that appropriate good manufacturing practices and good tissue practices are followed and that corrective action is taken as necessary, develops and maintains the quality plan, and ensures that quality objectives and policies are clearly defined.

- The CPS Section Chief/Medical Director ensures that quality policies and practices are incorporated into the procedures of the section, ensures that all employees receive adequate training in good manufacturing practices and good tissue practices and in the duties of their positions, and directs operations and resource planning efforts.

- The CPS Technical Supervisor manages the implementation of quality assurance practices, provides appropriate training to staff, ensures that operational policies and procedures comply with regulatory and accrediting agency requirements, and monitors and reviews systems in the section and initiates corrective actions when necessary.

- The CPS Quality Coordinator designs practical systems for the section's quality program, coordinates development and implementation of CPS policies and SOPs, serves as responsible party for CPS document control, coordinates preparation of data and documents for CPS audits and inspections, and serves as liaison from CPS to the DTM Quality Assurance Committee.

C. Personnel Training and Competency Assessment

Training and competency assessment are carried out according to DTM-SOP-0003 (Training Personnel in DTM Standard Operating Procedures), DTM-SOP-0015 (DTM Policy on Training and Education), DTM-SOP-0024 (Training Manual), and DTM-SOP-0007 (Competency Assessment). These procedures are designed to assure that work activities are performed only by those qualified to perform them, and that employees are educated on the consequences of improper performance of their assigned responsibilities. Records of personnel training and competency are maintained in CPS files.

D. Other Elements and Functions of the Quality Program

1. Procedures and Document Control

Standard operating procedures (SOPs) are managed according to DTM-SOP-0001 (Preparation and Maintenance of Standard Operating Procedures) and DTM-SOP-0002 (Validation and Implementation of New Standard Operating Procedures). Prior to implementation, SOPs are reviewed and approved by the DTM QS Officer and DTM Department Head (for department-wide SOPs) or by the CPS Technical Supervisor and CPS Medical Director (for CPS-specific SOPs).

2. Procedure and Process Design

Procedures and processes are designed to ensure consistency in manufacturing and to prevent errors that may compromise product integrity or function, or lead to transmission of adventitious agents. PIs participate with CPS in the development and approval of final process design specifications for clinical cellular therapy product manufacturing. Process design, process control, and process change control are further described in Section VIII (Manufacturing Systems and Process Controls).

3. Records Management

Records are managed according to DTM-SOP-0016 (DTM Record Keeping Policy). This policy is based on the US Food and Drug Administration (FDA) regulations and the guidelines of NIH, the American Association of Blood Banks

(AABB), the Centers for Medicare and Medicaid Services (CMS), and the American Society of Histocompatibility and Immunogenetics (ASHI). The policy is also designed to promote compliance with the Privacy Act (1974), the Freedom of Information Act (1966), and the Health Insurance Portability and Accountability Act (1996).

Records are maintained concurrently with the performance of each significant step in product manufacturing (including recovery, processing, storage, labeling, packaging, and distribution), donor screening and testing, and with the performance of significant activities involving the facility, equipment, supplies, and reagents.

Manufacturing records are currently retained as paper records, with a retention period of at least 10 years after the date of product infusion, distribution, or disposition (whichever is latest).

Records of agreements with external parties for assays or other contract services are maintained in CPS. The NIH clinical protocol and protocol-specific requirements, described in Section VIII (Manufacturing Systems and Process Controls), constitute agreements with Institute investigators to manufacture products for specific protocols.

4. Quality Control Procedures

   Regularly recurring quality control (QC) procedures are performed by CPS staff on a rotating schedule. The CPS QC schedule for daily, weekly, and monthly QC procedures is provided in **Attachment 3.** Other detailed QC procedures are incorporated into written procedures for equipment operation, test systems, and manufacturing processes.

5. Management of Deviations, Adverse Events, and Customer Complaints

   a. General

      Management of incidents is performed according to SOP-DTM-0017 (Reporting and Resolving Errors, Accidents, and Deviations from Standard Practice). This process includes:

      - Recognition and reporting of the incident
      - Investigation and root cause analysis of the incident
      - Corrective action aimed at achieving process improvement, error prevention, and/or improved customer satisfaction.

The DTM QS Officer and CPS Quality Coordinator determine the need for quality improvement projects based on the nature and extent of incidents reported.

b. Communication of Incident, Product Deviation, Adverse Event, and Customer Complaint Reports

CPS, DTM, and the CC have multiple and overlapping mechanisms for generating and receiving reports of incidents, product deviations (including errors, accidents, and nonconformances), adverse events, and customer complaints. All such reports are managed under the same incident reporting system described above. The mechanisms include:

- CPS SOPs, forms, checklists, and record keeping practices are designed to detect errors and omissions in manufacturing
- CPS staff performing manufacturing or clerical checking functions are trained to recognize and report errors and incidents
- CPS supervisory staff perform reviews of manufacturing and quality records by a structured process designed to detect errors and incidents
- CPS and DTM medical staff are required to review pertinent manufacturing records during exceptional release of products
- CPS or DTM medical staff maintain an active consultation service with clinical care staff and are trained to receive and investigate patient adverse events suspected as related to product infusion
- CPS Clinical Service Coordinator and/or DTM medical staff attend regular weekly meetings with the 2 major transplant services (NCI and the National Heart, Lung, and Blood Institute (NHLBI)) during which patient outcomes are systematically reviewed
- DTM QS Officer receives reports from the web-based CC Occurrence Reporting System, designed to capture, report, and track unexpected events in the hospital environment
- CPS staff, CPS Technical Supervisor, CPS Clinical Service Coordinator, and CPS Medical Director receive customer complaints from the protocol PI or clinical care staff

6. Management of Information or Test Results Received after Product Collection, Manufacture, or Distribution

Management of reports of donor information that might affect the integrity or function of a cellular product, or result in possible contamination of the product or the potential transmission of communicable disease by the product is handled according to DTM-SOP-0023 (Management of Postdonation Information Reports). Positive sterility (bacterial and fungal) test results or other abnormal test results received after distribution of the product are handled according to action plans described in Section IX (Product Evaluation and Lot Release).

7. Reporting Deviations and Adverse Events to the PI and FDA

   The role of CPS in reporting product deviations and adverse events is to notify the NIH protocol PI about the deviation or event in a timely manner. In addition, CPS keeps appropriately detailed records so that these deviations can be included in the manufacturing summary for IND annual reports or for further investigation as appropriate. Product deviations and patient adverse events are reported to FDA by the NIH Protocol PI, within specified timeframes, after assessment of the severity of the event and its potential consequences to the recipient.

8. Formal Assessments, Inspections, and Audits

   a. Internal Assessments

      Regular internal assessments or audits are conducted by the DTM QS Officer, who does not have direct responsibility for the processes being audited. Internal assessments include system-wide and targeted audits of quality program and operations, direct observation of staff performance, annual review of standard operating procedures, monitoring of error reports, and monitoring of deviations from standard practice.

   b. External Assessments

      FDA establishment registration for HCT/Ps was added to our existing establishment registration for Blood and Blood Products on January 24, 2002 (Establishment name: National Institutes of Health Warren Grant Magnuson Clinical Center, Registration number 1174694).

      Regular external assessments or audits of the DTM are conducted by the US Food and Drug Administration (FDA), the American Association of Blood Banks (AABB), and the Joint Commission on Accreditation of Health Care Organizations (JCAHO). Dates of most recent assessments and audits will be provided as an attachment to the Master File transmittal letter, and in annual updates.

9. Quality Improvement

In addition to monitoring corrective and preventive actions, all deviation and incident report information is reviewed, summarized and trended by the QS Officer to identify opportunities for improvement. The results of assessments, performance monitoring activities, and customer complaints may also serve as the impetus for initiation of a quality improvement project. Once identified, a quality improvement effort is managed using the Plan Do Check Act (P-D-C-A) principles.

III. Facilities

A. General Information

1. The CPS facility, located on the 3rd floor of Building 10 on the NIH campus, was designed and constructed in 1997 to accommodate expanding service demands and to promote compliance with evolving standards and regulations for cellular therapy products. The NIH Design Policy and Guidelines, developed by the NIH Division of Engineering Services, provide minimum criteria for design of CC facilities.

2. The floorplan of the facility is shown in **Attachment 4**. The facility consists of a 2200 ft^2 manufacturing space, a 700 ft^2 administrative support area, and a 400 ft^2 laboratory annex. The manufacturing space consists of contained areas (a material receiving room, storage rooms, toilet, lockers, gowning/entry room, and a refrigerator/freezer room), and areas for aseptic processing (two cell separation and processing spaces and centrifuge area within one large space, two discrete tissue culture rooms, and a flow cytometry room). The laboratory annex is used for housing incubators and storage of clean supplies and equipment.

3. Flow patterns within the facility for personnel, products and materials, and waste are shown in **Attachments 5, 6, and 7,** respectively.

4. The facility's manufacturing space and the administrative support area are served by a single air supply. The laboratory annex is served by a separate air supply. The details of the air supply are provided below in the section on HVAC. Air pressure differentials and airflow between adjacent areas of the facility are shown in **Attachment 8**.

5. Construction features of the manufacturing area are as follows. All walls and ceilings are constructed of solid wallboard with epoxy coatings. The floors are constructed of seamless, welded vinyl with integral coving at all walls and corners. Work surfaces in the manufacturing area are smooth and non-porous. All penetrations, including lights, fixtures, filters, switches, power and gas outlets, are sealed with silicone caulk or similar material.

B. Water Systems

1. Within the general manufacturing area there are 4 sites where tap water is used, each consisting of two faucets with sink and drain. Three are located within the common separation and processing spaces, and one is located in the flow cytometry room. There are no sinks and drains located in either of the two tissue culture rooms.

2. Sterile water required for manufacturing is purchased.

C. Heating, Ventilation, and Air Conditioning (HVAC)

1. In the main manufacturing area and administrative support area, the HVAC system was designed to provide a continuous supply of filtered air that is maintained within specified temperature and humidity ranges. Additional features are as follows:

   - The system was originally designed to meet class 100,000 requirements, but the final engineering specifications have resulted in a class 10,000 environment.

   - External air is drawn into rooftop vents and fed into two air handling units, each with its own high efficiency particulate air (HEPA) filter; these two units feed into a single duct for delivery of air to the facility.

   - The airflow is unidirectional and exhausted from the facility after a single pass (i.e., it is not recirculated) at a rate that allows for at least 20 air changes per hour.

   - Air pressure is maintained at different levels throughout the facility's various rooms and areas. Positive pressure is maintained in most areas of the manufacturing space, with the culture rooms having higher positive pressure than the other areas. Negative pressure or "sink" areas include the gowning/entry room, the refrigerator/freezer room, the material receiving room, and the storage rooms.

2. The HVAC system in the laboratory annex is separate from the HVAC system of the main manufacturing and administrative support areas, and was designed to meet general NIH CC laboratory specifications for providing a continuous supply of air that is maintained within specified temperature and humidity ranges.

D. Facility Maintenance

Preventive maintenance of the facility is performed by the Maintenance Department of the NIH Clinical Center. Computer controls and monitoring systems for the facility's HVAC system are described in Section IV (Environmental Control and Monitoring). Certification of all biologic safety cabinets is performed on a twice yearly basis by the NIH Division of Safety.

E. Cleaning and Sanitation

1. Cleaning and sanitation practices are guided by the NIH CC Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Exposure Control Plan, and also by procedures designed to minimize particulates in the environment.

2. The NIH CC Housekeeping Department performs regular cleaning and sanitation procedures for the facility. These include:

    - Removal of all trash (solid waste), including medical-pathological waste, from the facility on a daily basis.

    - Cleaning of floors in the manufacturing space on a daily basis, and in the administrative areas on a weekly or as needed basis, using an Environmental Protection Agency (EPA)-registered hospital disinfectant (List B) approved by the NIH CC Infection Control Committee.

    - Additional cleaning of floors, walls, and ceilings in the manufacturing space on an as-needed basis.

3. CPS technologists perform additional cleaning procedures, including:

    - Cleaning of countertops in the manufacturing space, on an as-needed basis, with soap and water.

    - Cleaning of sinks in the manufacturing space on a weekly basis.

    - Clean up of spills, including clean up of hazardous materials, on an as-needed basis, according to the NIH CC OSHA Bloodborne Pathogens Exposure Control Plan.

4. Biologic cabinets are cleaned, decontaminated, and certified on a regular basis, as follows:

    - Monthly cleaning, performed by CPS technologists, includes thorough scrubbing with detergent and water, followed by cleaning with 70% ethanol, of all interior cabinet surfaces, including the airflow path beneath the grill.

    - Every 6 months, the NIH Division of Safety supervises the decontamination of each cabinet with paraformaldehyde, and certifies that the cabinet's physical parameters (airflow, and pressure required to push air through HEPA filter) are within defined specifications.

    - Cleaning and decontamination before each production run is done by wiping the work surface with 70% ethanol, followed by complete air-drying.

5. Equipment within the facility is regularly cleaned using procedures described in CPS Quality Control SOPs, listed in Section VI (Equipment, Supplies, and Reagents). Surfaces that come into contact with products, either intentional or inadvertent, are subject to cleaning, disinfection, and sterilization procedures, after such contact, according to the NIH CC OSHA Bloodborne Pathogens Exposure Control Plan, instructions in operator's manuals, and DTM SOPs.

    - The Beckman elutriation rotor and chamber, used for elutriation by a method that is not considered closed, are cleaned and sterilized between production runs as follows. Before each run, the rotor, chamber, and integral silicone tubing are sterilized with a 40% bleach solution, followed by 70% ethanol, followed by sterile water. After each run, the rotor and chamber are decontaminated with 40% bleach, followed by sterile water, and then disassembled and cleaned with detergent and water.

F. Containment Features

In addition to the physical plant features described above (i.e., specific contained areas surrounding the core manufacturing space and the airflow and air pressure differentials), which address overall facility containment and the prevention of contamination and cross-contamination of products, Section V (Operational Control Systems and Aseptic Processing) describes practices used within the facility for containment and segregation of products.

IV. Environmental Control and Monitoring

A. Environmental Control

Areas of the manufacturing space within the facility are classified as either critical areas or supporting clean areas.

Critical areas include the 6 biologic safety cabinets (2 in each of the tissue culture rooms, 2 in the common separation & processing areas). These areas are designed and controlled so that Class 100 requirements are met. All open system processing steps occur in these areas.

Supporting clean areas include all other areas of the manufacturing space, including areas surrounding the biologic cabinets. These areas are designed and controlled so that Class 10,000 requirements are met. Closed system processing steps are performed in these spaces.

B. Environmental Monitoring

Environmental monitoring consists of systems for ongoing control and monitoring of the HVAC system and actual quantitation of particulates in all areas of the facility, to ensure that specifications are met for class 100 in the critical areas and class 10,000 for the supporting clean areas.

There are three systems used for ongoing control and monitoring of the HVAC system that are managed by the NIH Office of Research Services. These include:

- The HVAC dedicated air handling unit is controlled and monitored by a direct digital computer (DDC) balancing system.
- A stand-alone computerized system is used to provide ongoing monitoring of the facility for temperature, humidity, pressurization, supply and exhaust airflow, and rate of air changes, with parameters measured every 15 minutes, 24 hours/day, every day. These data are captured in a computerized database.
- A twice-yearly survey is performed to measure air volumes (supply and exhaust), with calculation of air flows and rates of air change in all areas of the manufacturing space.

In addition to systems used to control and monitor the HVAC system, quantitation of nonviable particulates in the facility is performed twice a year. Reports are reviewed by the CPS Quality Coordinator to ensure that particle counts fall within specified limits.

V. Operational Control Systems and Aseptic Processing

A. Operational Control Systems

Although the CPS facility has several areas and rooms designated for specific activities, it was designed for simultaneous handling of multiple products of different types and from different donor sources within the same manufacturing space. Specific measures and control systems have been established to prevent improper labeling, mix-ups, contamination, cross-contamination, and accidental exposure of products to communicable disease agents. These systems include labeling systems, product segregation and containment systems, and policies for cleaning biologic cabinets and other equipment between production runs.

1. Labeling systems are described in Section XI (Product Labeling, Label Controls, and Tracking).

2. Product segregation and containment systems are based on the following policies:

   - Xenogeneic cells and tissues are not handled or stored in the CPS facility.
   - Products from donors with confirmed positive testing for HIV are not handled or stored in the CPS facility.
   - Products from donors with evidence of hepatitis B or hepatitis C infection are not handled or stored in the CPS facility without review and approval of the CPS Medical Director.
   - For products cryopreserved and stored in bags or vials as the primary container, plastic overwrap bags are used as a 2nd layer of containment during storage and thaw.
   - Cryopreserved products are held within the liquid nitrogen (LN2) storage tank, on top of the racking system (in vapor phase) if donor transfusion-transmitted disease (TTD) prescreening has not been performed, is incomplete, or is awaiting confirmatory testing.
   - Products collected from donors with repeat reactive testing and positive confirmatory testing for TTD screening tests are stored in a separate freezer.
   - Only one product is handled at a given time in a given biologic cabinet.
   - Products from more than one patient may not be held in a given incubator at any given time.

B. Aseptic Processing Methods

Closed system processing steps are used whenever technically feasible, and take place within the supporting clean areas. Single use, disposable plasticware is used in most situations. All open system processing steps are performed using strict aseptic technique in the critical areas (i.e., biologic safety cabinets). Aseptic processing policies and methods are described in DTM-SOP-5036 (Use of the Biologic Safety Cabinet/Aseptic Technique), which addresses the following:

- Staff attire and gowning
- Use of biologic cabinets
- Manipulation of sterile reagents and materials in closed and open systems
- Transfer of cells, media, reagents between containers in closed and open systems
- Sampling of cells, media, reagents, materials

In addition to defined training and competency assessment of staff in aseptic methods, the appropriate use of aseptic methods is ultimately monitored by sterility testing on all final products. These assay data are reviewed and investigated for individual products and for ongoing trends.

VI. Equipment, Supplies, and Reagents

A. General Policies and Systems

The following general policies and systems apply to equipment, supplies, and reagents used by CPS in the manufacture of cellular therapy products:

1. Evaluation of new equipment or supplies, or any new use of existing equipment or supplies, is performed by the CPS Technical Supervisor. This evaluation consists of a review of the vendor's qualifications as well as validation, as described in DTM-SOP-0005 (Installation and Validation of New Equipment).

2. When equipment is placed into service, procedures for the maintenance, repair, and periodic or post-repair calibration of equipment are implemented and documented.

3. For critical equipment, supplies, or reagents (i.e., those that come into physical contact with the product):

    a. Vendors are evaluated and selected for their ability to provide equipment, supplies, and reagents that consistently meet manufacturing requirements

    b. Whenever possible and/or indicated, items selected are sterile, disposable, and FDA-approved for human use

    c. Use in manufacturing is recorded in a manner to facilitate tracking of the individual item or lot

4. Equipment, supplies, and reagents are stored in a safe, sanitary, and orderly manner.

5. At the time of receipt, supplies and reagents are inspected for acceptability and logged into CPS inventory. Separate receipt logs are maintained for reagents, disposable supplies, and miscellaneous items. These logs include the following elements:

    - date received
    - manufacturer
    - item name/size
    - lot number
    - expiration date
    - number of units or boxes

- indication of acceptance
- initials of CPS technologist receiving item(s)

B. Equipment

1. Validation

Validation is performed on equipment according to validation plans designed to demonstrate that the equipment will perform consistently to produce the expected result.

a. For all new equipment, prospectively designed validation plans include three phases:

- Installation qualification (IQ), typically documented by the vendor representative trained to install equipment at the site of intended use

- Operational qualification (OQ), for which test cases or scenarios are developed to demonstrate that the equipment functions on site in the manner described by the vendor

- Performance qualification (PQ), for which test cases are developed to demonstrate that the equipment is capable of performing its intended function. For cell processing equipment, these test cases typically include handling of materials or cellular products of known content, and they are sometimes performed in parallel to equipment already in use. Acceptance criteria are developed prior to PQ testing of the new equipment.

If variances from expected results (i.e., failure to meet acceptance criteria) occur during any of the three phases, a root cause analysis is done, corrective actions are implemented, and the validation is repeated. Training of staff on new equipment is carried out after completion of validation.

b. Retrospective validation plans are designed, carried out, and documented for equipment installed and placed into service in the past, prior to implementation of DTM/CPS equipment validation requirements.

2. Maintenance, Repair, Calibration, and Cleaning

Procedures and schedules for maintenance, repair, calibration, and cleaning of equipment are established at the time of equipment installation. The following equipment QC and maintenance SOPs have been established in CPS:

DTM-SOP-5050	CPS QC Program
DTM-SOP-5051	Refrigerator QC

DTM-SOP-5052	Freezer QC
DTM-SOP-5053	LN Freezer QC
DTM-SOP-5054	Monitoring the Main LN Tank
DTM-SOP-5055	Biologic Safety Cabinet QC
DTM-SOP-5056	Waterbath QC
DTM-SOP-5057	CO2 Incubator Maintenance and QC
DTM-SOP-5065	Sterile Connecting Device QC and Servicing
DTM-SOP-5058	Centrifuge QC
DTM-SOP-5063	Maintenance of Eyewashers
DTM-SOP-5059	Maintenance of Sinks
DTM-SOP-5060	Monitoring of Disposal Sharps Containers
DTM-SOP-5061	Timer Calibration
DTM-SOP-5062	Thermometer and Pipette Calibration
DTM-SOP-5064	CS3000 Blood Cell Separator QC
DTM-SOP-5066	CellDyn Maintenance
DTM-SOP-5088	QC of Volumetric Dispensers and Use of HandyStep Repetitive Pipette
DTM-SOP-5094	Weekly, Quarterly, and Annual Maintenance and Data Storage or EPICS XL Flow Cytometers

3. Gamma Irradiation Equipment

Gamma irradiation equipment and procedures are detailed in DTM-SOP-3508 (Irradiation of Blood Components for Transfusion). Gamma irradiation is performed using the IBL 437 C Blood Cell Irradiator, which is fully automatic and driven by an electromechanical system. The timer display screen reads the currently calibrated time interval for exposure to the radioactive source, to deliver the specified dose to the midplane of the canister. The current dose for blood, plasma, and cellular therapy products (when specified in the manufacturing process) is 25Gy (midplane dose).

Preventive maintenance and calibration of the irradiator is performed annually by the CIS-US contract services. Dose validation and dosimetry mapping is also performed annually by CIS-US. Calculation of the irradiation source dose and medication to time of exposure is performed biannually. Records of these calibrations and measurements are kept in the IBL 437 C Blood Cell Irradiator operator's manual, located in DTM. Changes to the timer necessary to assure delivery of 25Gy to the midplane of the canister and a minimum of 15Gy to any other location in the canister are posted on the front of the irradiator.

Quality control to ensure delivery of the specified dose to the irradiated products includes checks of the irradiator timer and irradiator turntable checks, performed daily, and QC of RAD-SURE labels daily and with each new lot put into use, as described in DTM-SOP-3909 (Use of RAD-SURE Type 25 indicator). Safety

testing, and record keeping for maintenance and training and safety testing, are performed by the NIH Radiation Safety Branch (RSB).

C. Supplies

Whenever possible, sterile single-use disposable supplies are used. All items are obtained from qualified vendors.

D. Reagents

1. Critical Reagents

Most reagents used during the manufacture of CPS clinical products are considered critical, in that they come into contact with the product during manufacturing or are used in the final product formulation. Critical reagents that are FDA-approved for human use are used whenever available; these reagents (which include drugs and I.V. solutions) typically have package inserts available. When an FDA-approved reagent is not available, a vendor is selected who can provide a GMP-grade reagent or one that can be qualified for use in manufacturing. Specifications for such reagents are included on a Certificate of Analysis (COA) provided by the manufacturer.

The NIH CC Pharmacy Development Service (PDS) plays a role in formulating, re-formulating, or re-packaging of certain critical reagents and media used in the manufacture of CPS products. This is typically done so that the dosage, concentration, or aliquot size is more compatible with, or economical for, the product manufacturing schema. Certain critical reagents not considered GMP grade by the original vendor are handled, tested, and qualified by the PDS.

2. Critical Reagents From Human and Animal Sources

a. Human Sources

Critical biologic reagents from human sources used in manufacture of CPS clinical products include human plasma, human serum, and human serum albumin (HSA). Qualification activities include:

1) Human plasma and serum, obtained from whole blood or apheresis collections:
   - Autologous and allogeneic donors are screened as autologous and allogeneic blood donors, respectively, as described in Section VII (Donor Selection and Eligibility).
   - Allogeneic donors are either family-related or unrelated AB, Rh-negative male donors who have never been transfused and have a negative HLA-antibody screen.

- All final plasma and serum products are negative for bacterial and fungal testing, as described in Section IX (Product Evaluation and Lot Release) and Attachment 11-B.

2) Human serum albumin (HSA) is purchased as an FDA-approved biologic reagent from commercial manufacturers that follow FDA-required screening procedures for donors of human source plasma. A package insert is available for each lot of HSA used by CPS.

b. Animal Sources

1. CPS does not currently use ruminant materials (fetal bovine serum, fetal bovine serum albumin, bovine collagen, bovine-derived enzymes, or gelatin) as critical reagents for CPS clinical products, with the following exceptions:

   - Manufacture of viral vectors used for gene transduction may use fetal bovine serum. These vector products are qualified by the manufacturer, with a COA issued to the PI and CPS and included in the IND application.

   - Paramagnetic beads (Dynabeads, Dynal Biotech) with sheep antibody to mouse IgG are used for cell selection in some manufacturing processes. The antibody is obtained from sheep raised in Norway, a BSE-free country.

2. Critical reagents from non-ruminant animal sources include monoclonal antibodies from murine sources. These antibodies are qualified by the commercial manufacturer and/or by CPS, with the COA issued to CPS for each lot and included in the IND application.

3. Non-Critical Reagents

Non-critical reagents include those used for in-process or final product testing, e.g. for flow cytometry. Flow cytometry reagents are selected, received, validated, and managed according to DTM-SOP-5093 (Reagent Selection, Receipt, Validation, and Inventory for Flow Cytometry). When possible, these reagents are purchased from a commercial supplier and meet the classification of "analyte specific" or "for in vitro diagnostic use." For some assays, it may be necessary to use a reagent classified as "for research use only." When analytical and performance characteristics of the reagents are not established by the manufacturer, the CPS flow cytometry laboratory establishes performance characteristics of the reagent in the test system. Performance checks are done on all new lots of reagents, consisting of comparative testing with the previous lot and titration studies, if indicated.

VII. Donor Selection and Eligibility

A. Types of Donors for CPS Cellular Therapy Products

The majority of donors of cells and tissues for CPS cellular therapy products are living donors, most of whom undergo apheresis procedures in the DTM Dowling Apheresis Clinic. A small number of donors have bone marrow harvested by percutaneous aspiration in the surgical operating rooms.

Living donors are either autologous patient-donors or family related allogeneic donors. CPS does not currently support protocols involving cells or tissues from unrelated living allogeneic donors for manufacture into cellular therapy products.

Cadaveric donors are used as a source of pancreata used for manufacture of pancreatic islets.

B. Donor Selection

Selection of donors for cellular therapy products manufactured or handled by CPS is guided by criteria outlined in IRB-approved clinical protocols and is the responsibility of the protocol PI. These are typically outlined in each protocol as inclusion and exclusion criteria. The tables in **Attachment 9 (A-D**) outline specific measures and procedures for each donor type.

C. Donor Screening for Transmissible Disease

Both donor health history and blood testing are used to screen donors for transmissible disease.

The donor health history questionnaire used for screening living donors of cellular therapy products is the same as that used for screening blood donors by the NIH Department of Transfusion Medicine. This questionnaire is updated regularly to be in compliance with FDA regulations and AABB Standards. The following special considerations with regard to donor screening are noted:

1. The questionnaire includes questions about risk factors or clinical evidence of relevant communicable disease agents and diseases.

2. The questionnaire includes questions about risk factors or clinical evidence of TSE, including CJD. However, potential CJD risk based on travel history is generally not a reason for rejection of related allogeneic donors who have been selected on the basis of HLA matching with a genetically related

recipient.

Testing of donor blood for transmissible diseases is performed by the DTM and the CC Department of Laboratory Medicine (DLM). Results are reviewed by CPS staff. The following special considerations for donor blood testing are noted:

1. All cells and tissues used for CPS products are considered to be leukocyte-rich; therefore all allogeneic donors are tested for HTLV-I/II and CMV.

2. Although not required by FDA, all autologous donors for cellular therapy products undergo the same blood testing as living allogeneic donors, with the exception of CMV.

D. Acceptance of Donors and Products Based on Selection and Screening Criteria

Criteria for acceptance or rejection of a donor or product based on transmissible disease screening are outlined in the tables in **Attachment 9 (A-D)**.

E. Documentation of Transmissible Disease Screening

Policies for documentation of transmissible disease screening are outlined in the tables in **Attachment 9 (A-D)**.

F. Notification Process for Abnormal Transmissible Disease Screening Results

Policies for notification for abnormal transmissible disease screening results are outlined in the tables in **Attachment 9 (A-D)**.

VIII. Manufacturing Systems and Process Controls

A. Defined Manufacturing Processes

1. Process Design and Validation

For each type of cellular therapy product, the manufacturing process is developed, defined, and validated as follows:

a. Process development: Clinical-scale manufacturing processes are designed based on data from previous small-scale studies, desired product characteristics, anticipated results from processing methods appropriate to the manufacturing process, and use of clinical-grade systems, materials, and reagents. Developmental studies are performed to provide initial data upon which to base the final process design that will be validated for clinical use. Product specifications and test methods are refined during these developmental studies.

b. Process definition: At the end of developmental studies, the process is defined in terms of:
   - Special requirements for aseptic methods, environmental controls, or operational controls, if applicable
   - Special requirements for equipment, supplies and clinical-grade reagents
   - Starting source material
   - All active manipulation steps
   - All holding and storage steps
   - In-process testing and controls
   - Final product testing and specifications

c. Process validation: After the process is developed and defined, it is validated, typically in a series of 3 runs. These runs are performed at clinical scale, with starting material, equipment, supplies, and reagents identical to or comparable to those used for manufacture of the clinical product. Failure to meet anticipated results and/or product specifications in validation studies typically leads to additional development work, re-definition of the process and/or product specifications, and repeat validation studies.

d. Process implementation: Once validated, the process is implemented by finalizing all appropriate SOPs and forms, training staff, and obtaining approvals from the CPS Medical Director and NIH protocol PI.

e. Process monitoring: After implementation, manufacturing processes are monitored on an ongoing basis by supervisory review of production and quality management records, as well as product deviation and adverse event reports, and the complaint file.

2. Process Change Control

Once implemented, a defined manufacturing process can be changed only by the following mechanisms:

a. Permanent changes to the process are made only after appropriate verification or validation, to ensure that the change does not adversely impact the product characteristics or the overall operation. The new process must be implemented in a manner similar to that described for initial process validation.

b. Non-permanent changes to the process, i.e., changes made for an individual product, are made only by exception, typically in situations where a patient-specific product must be prepared from suboptimal starting material or after unexpected events have occurred during manufacturing. In such cases, there must be a documentation of the rationale, the process steps planned, the results of processing, an assessment of the anticipated risks and benefits to the recipient, and the signed approvals of the CPS Medical Director and the NIH protocol PI. These exceptions to defined manufacturing processes are documented in the process record. Exceptions to defined manufacturing processes are reported to the IRB and FDA, if indicated, and are summarized in annual IND reports.

3. Documentation of Defined Manufacturing Processes

a. SOPs contain instructions on performing manufacturing processes, or portions of those processes.

b. Protocol-specific requirements are developed for each clinical protocol, which may involve the manufacture of one or more product types. The protocol-specific requirements define what products are to be manufactured for a given clinical protocol and by what SOPs. Each protocol-specific requirements document is approved by the CPS Medical Director and the NIH protocol PI prior to implementation of each clinical protocol.

B. Medical Order for Manufacture of Patient-Specific Products

A written and signed physician's order is obtained prior to manufacture of each patient-specific product. The order contains identifiers of both the donor and recipient and any exceptions to the manufacturing process previously defined in the protocol-specific requirements.

C. Cell Manipulation Procedures

CPS has SOPs for a range of ex vivo cell manipulations, including cell separation and isolation, cryopreservation, cell culture, and immunologic and genetic manipulations. These manipulations may be combined in a series of steps for a given manufacturing process, which is defined in protocol-specific requirements and the individual IND application.

Cell separation and selection procedures widely used in the manufacture of cell therapy products by CPS are listed in **Attachment 10**; the majority of these would be considered minimal manipulation.

Advanced or more complex methods for ex vivo manipulation of cells, including cell culture and expansion, immunologic manipulations, and gene transduction, are always done in the context of IND-related clinical trials, and are therefore described in detail in individual IND applications. Some complex processes may incorporate cell separation methods mentioned above, and in Attachment 10.

D. Cryopreservation Methods

Prior to October 1, 2000, CPS products were cryopreserved in a solution that resulted in a final concentration of 10% DMSO. Since October 1, 2000, CPS products have been cryopreserved by a validated procedure that results in final concentrations of 5% DMSO and 6% Pentastarch, as detailed in IND# 9164. Following the addition of the freeze solution to the cell suspension in a 1:1 ratio, the cellular mixture is transferred to the final freezing containers, either bags or vials, and placed in a controlled-rate device. Frozen bags or vials are then placed and sealed into a plastic overwrap bag and transferred into an aluminum cassette for placement into the racking system of a liquid nitrogen (LN2) storage tank. Products are stored in either the liquid or vapor phase of LN2.

Following the observation and investigation in the CPS facility of a series of catastrophic failures of Cryocyte (Baxter) freeze bags that began in January 2000 (Khuu et al. Cytotherapy 2002;4:539-549), CPS validated and implemented the use of KryoSafe freeze bags (American Fluoroseal), beginning in January 2002.

E. Gamma Irradiation

Gamma irradiation is performed according to DTM-SOP-3508 (Irradiation of Blood Components for Transfusion) using a Cesium^{137} source contained in the CIS IBL 437 Blood Cell Irradiator. The DTM holds an FDA-approved BLA #103044 for irradiation of blood products. RAD-SURE 25Gy Blood Irradiation Indicators are used to provide positive visual verification that a product has been irradiated with the specified dose of 25Gy, according to DTM-SOP-3909 (Use of RAD-SURE 25Gy). Prior to exposure,

the word “NOT” is visible in the window of the indicator. After irradiation, the word “NOT” should be obliterated and the indicator should read “IRRADIATED.” Details of the equipment, maintenance, QC, and safety procedures associated with gamma irradiation are provided in Section VI (Equipment, Supplies, and Reagents).

G. Containers, Connections, and Filters

A variety of containers, connections for transfer, and filters for cellular therapy source material, in-process products, and final products are used during ex vivo processing. All such supplies are sterile, single-use, and disposable, with the exception of specific containers used in pancreatic islet processing that are re-sterilized before each use.

IX. Product Evaluation and Lot Release

A. Donor and Product Testing

1. Testing of donor blood and products is performed either in-house or by other testing facilities. Assays include those that address product safety, dose, identity, potency, or purity.

2. Assays may be done for in-process testing or for final product testing. Release testing refers to the performance of assays whose results are required before the product is released from quarantine status and made available for distribution.

3. The assays described in the tables in **Attachment 11** constitute those that are not specific to a given product or IND. Product-specific assays, with definition of acceptable results or limits, are described or listed in individual IND applications and on the certificates of analysis (COAs).

4. Action plans for abnormal results are outlined in the tables in **Attachment 11**. In general:

   - Assay results are initially communicated to CPS personnel, and critical abnormal results are communicated to the PI, within timeframes determined by how critical the assay/result is and whether the product has already been infused or still in storage.

   - It is the PI's responsibility to notify the product recipient, the IRB, and the FDA, if indicated, of the abnormal test result, and to determine what medical interventions are to be performed.

   - Abnormal or unexpected results are investigated in a manner that depends on the nature of the result and whether it may be an indicator of a problem in the manufacturing process or the donor.

B. Lot Release Procedures

Each product or product lot is released from quarantine status only after the following are documented, reviewed, and judged as passing defined lot release criteria:

Donor screening & testing

- Summary of donor health history screening results (day of collection)
- Donor transmissible disease testing (donor blood from day of collection)
- Donor ABO/Rh and RBC antibody screen (donor blood from day of collection)

Product testing

- Total cell content of product
- Volume of product
- Sterility testing
- Other assays as defined in CPS protocol-specific requirements that address safety, dose, identity, potency, and/or purity

Manufacturing Records

- Comprehensive clerical review for completeness and accuracy and to identify any irregularities that need to be addressed or resolved
- Review of critical calculations

C. Exceptional Release of Products

Products may be released from quarantine and made available for distribution by exception to standard lot release procedures under the following circumstances if the product is needed for clinical use because of protocol requirements (e.g., family-related allogeneic use and/or timing of therapy) or for urgent medical need prior to completion of release testing or in the face of one or more abnormal tests. Steps for exceptional release are as follows:

1. All routine lot release procedures (indicated above) are completed, including entry of all available test results and initial record review.

2. The final review of test results and records is performed. Any incomplete or abnormal test results are flagged.

3. The relevant portions of the product record, with incomplete and/or abnormal test results, are reviewed by the CPS Medical Director.

4. When indicated, the CPS Medical Director discusses the incomplete and/or abnormal test result(s) with the PI, who decides if exceptional release of the product is warranted.

5. In situations where the incomplete and/or abnormal test result(s) may be associated with potential risks to the recipient, it is the PI's responsibility to discuss the result(s) with the recipient and obtain informed consent, if indicated.

6. Approval of exceptional release of the product is documented on the product record, with a notation of the incomplete or abnormal result(s) and the signed approval of the CPS Medical Director.

X. Storage

A. Control of Storage Areas

Products, supplies, reagents, and equipment are stored in designated areas, with access limited to authorized personnel.

All products and materials in storage are contained, labeled, and organized in a manner designed to prevent mixups, commingling, deterioration, contamination, and cross-contamination, to prevent adverse effects on the function or integrity of the products, and to prevent improper release of products for distribution.

Products are stored within the main CPS manufacturing facility in the area designated as the “refrigerator/freezer room” on descriptions of the CPS facility.

B. Storage Temperature and Conditions

For each product type, the storage temperature and conditions are defined for products and materials. Five general categories of storage temperatures/conditions are used in CPS, and these are monitored, recorded, and reviewed in a manner to ensure that acceptable limits have not been exceeded. The categories are:

- Frozen storage, liquid nitrogen, liquid (-196°C) or vapor phase (-120°C to -145°C)
- Frozen storage, -70°C freezer
- Frozen storage, -20°C freezer
- Refrigerated storage, 2° to 8°C
- Room temperature storage, 18° to 24°C

All cryopreserved products stored in liquid nitrogen storage tanks (liquid or vapor phase) are contained in a primary container surrounded by a secondary plastic overwrap bag. These conditions are designed to prevent contamination and cross-contamination of products during storage and thawing procedures.

C. Product Expiration Dating

For each product type, shelf life and expiration dating are defined, and each product’s expiration date is designated on the label.

In the CPS facility, maximum storage period for cryopreserved products stored in liquid nitrogen (liquid or vapor phase) is indefinite, unless otherwise specified in the protocol. Products with indefinite storage periods are labeled with the collection date.

The following are current policies relevant to product expiration:

- Processing or storage at 2° to 8°C must be initiated within 4 hours of product collection
- PBSC and bone marrow products must be infused or cryopreserved within 24 hours of collection
- Cord blood may be stored in the liquid state at 2° to 8°C for up to 48 hours before processing or cryopreservation
- Lymphocyte products must be infused within 24 hours of collection, or cryopreserved within 12 hours of collection
- Mononuclear cell products destined for dendritic cell production must be processed within 24 hours of collection
- Cultured cells must be infused or cryopreserved within 4 hours of completion of harvest/concentration
- Thawed cells must be infused or placed into culture within 2 hours of the time the cells are removed from frozen storage

D. Freezer Inventory Management

Product freezer inventory records are maintained in a computerized database that contains fields for protocol, product type, product number, patient name, and freezer location. The freezer location of each product is also recorded on the product record. Internal and external audits have demonstrated that this system allows the rapid and accurate location of a given product.

XI. Product Labeling, Label Controls, and Tracking

A. Labeling and Label Controls

1. General procedures for labeling and label controls for CPS products are addressed in DTM-SOP-5016 (General Labeling Guidelines), and include the following:

    - Product labels are customized for each specific product type. For each customized label, the content and format is designed to comply with FDA regulations, and the label is approved by the CPS Medical Director and CPS Technical Supervisor before it is finalized and placed into service.

    - A copy of each label is kept in the Master label record which contains pertinent information on the label's computer file name, storage location of the label copies, use of the label, and dates of label use, editing, and removal from service.

    - A label generation log is maintained, and contains a record of the number of copies of each label printed and issued to a specific storage location. When a label is removed from service, the copies of the label are retrieved from the storage location and destroyed.

2. Specific labeling instructions for each product are included in the protocol-specific requirements.

B. Tracking

1. CPS has policies, procedures, and systems in place that enable

    - Tracking of all products from the donor to the recipient or final disposition: these procedures ensure that patients receive the products intended for them and also allow notification or investigation of the recipient of a product from a donor found to have an infectious disease or other condition that could potentially harm the recipient (e.g., lookback investigation).

    - Tracking from the recipient or final disposition of the products back to the donor of the source material: these procedures ensure that the donor can be identified and linked to the recipient when needed to investigate an adverse event in the recipient.

2. The tracking system in CPS includes procedures originally developed for tracking blood products, and is based on the following identifiers:

- Patient (recipient) identifiers: Each patient (recipient) is identified by both name (typically last, first, middle initial) and a unique medical record number (MRN). The MRN system is administered by the NIH Clinical Center.

- Product identifiers

  - Donation identification number (DIN): Each donation is assigned a unique DIN as soon as the initial collection (apheresis, bone marrow collection) takes place. The DIN assignment system is administered by the Department of Transfusion Medicine, according to DTM-SOP-0031. The DIN is used on all products, fractions, and aliquots derived from the original donor product.

  - Individual fractions and aliquots of products are uniquely identified by added extensions to the DIN (e.g. Bag A, Bag B,...).

  - For products collected or procured outside of the NIH Clinical Center, a unique DIN is assigned upon receipt in CPS, and any number previously assigned at the collection/procurement site is recorded in the CPS processing record. Linkage between the CPS-assigned number and the original collection/number is maintained in records, but not in labeling.

3. Use of the donation and recipient identifiers is guided by the following policies:

- For each product that is patient-specific, where the recipient has already been identified, both the recipient identifiers and the DIN are placed on the product labels, and on all aliquots, splits, and manipulated fractions, and samples of that product, through the final infused product. All product records, including assay records, contain both the DIN and recipient identifiers

- For products that are not patient-specific and/or for which the recipient has not yet been identified, the DIN is placed on the products, aliquots, splits, manipulated fractions, and sample labels and on all product records, including assay records.

4. Process control measures for this system to ensure that products can be tracked accurately include:

- All product processing steps and transfers, product container labeling steps, and documentation steps are performed by medical technologists specifically trained in labeling and documentation procedures.

- Every processing step is documented by the performing technologist on a processing checklist or process record specific for the protocol.

- Before transferring the product or sample into a new container, the technologist is required to label the new container.

- Clerical checking of labels is performed at pre-defined critical control points, with two trained persons verifying the accuracy and completeness of labeling information. The control points include (1) in the apheresis unit, at the time of pick up of the apheresis product by CPS staff, (2) in CPS, at the time the product is placed into storage, and (3) in CPS, at the time the product is removed from storage and prepared for issue to the patient care unit (this may include pooling of vialed products into a single container).

- There is a CC Department of Nursing SOP (Infusion of Products for Cellular Therapy), describing receipt and administration of the product on the clinical care unit. At the patient's bedside, the patient's name and medical record number on his/her hospital wristband are checked by two trained people (typically registered nurses) against the patient's name and medical record number on the product to be infused. The product is infused only after verification of a match between the recipient's identifiers on the product and the recipient's identifiers on the wristband.

- Completion of the infusion is documented by the infusing nurse by (1) placing a sticker tag (containing DIN, patient identifiers, and other information), originally attached to the product container, into the recipient's paper medical record and (2) documenting the details of the infusion and adverse events into the recipient's electronic medical record in the NIH medical information system (MIS).

XII. Product Receipt and Distribution

A. Receipt

Cell, tissue, and organ source material, cellular therapy products, and biologic samples for testing are received into the CPS facility by trained CPS technologists. Each incoming source material, product, or sample is inspected and the following data elements are recorded on a Product/Sample Receipt log:

- Date received
- Initials of person receiving product or sample
- Product or sample number/identifier
- For clinical (KS) products/samples: name and identifier/medical record number of recipient, if known
- For research (KR) products/samples: name and identifier of donor, if known
- Protocol or project number
- Name of product or sample type
- Appearance of product or sample
- Comments on appearance, if abnormal or irregular
- Name of tests or procedures to be performed on the product or sample

If any abnormalities or irregularities are observed in the packaging or appearance of the product or sample, the CPS Technical Supervisor and/or the Medical Director and/or the protocol PI are contacted for further actions and decisions about the disposition and further handling of the product or sample. If the product with unanticipated abnormalities or irregularities has been shipped from another facility, that facility is notified. Products that cannot immediately be accepted for handling or processing are placed into quarantine. Rejection of a product at the time of receipt is indicated on the log.

Outright rejection by CPS of received source material or cellular therapy products is an unusual occurrence. For products intended for patient-specific use that are considered difficult or impossible to replace, all possible efforts are made to salvage them even if they do not meet predefined specifications. Product salvage efforts are planned and approved by the CPS Technical Supervisor, CPS Medical Director, and the protocol PI, and documented as deviations to SOP.

B. Distribution

CPS distributes products in-house (i.e., to the NIH Clinical Center) and to off-campus sites. In-house distribution is considered “Issue” and is further described in Section XIII (Final Product Preparation, Issue, and Administration).

For products manufactured or stored by CPS, availability for distribution is determined on the basis of product/lot release procedures, as defined in Section IX (Product Evaluation and Lot Release). Documentation of distribution for these products is done in the computerized Soft Bank system.

Products manufactured or handled by CPS to be distributed off-campus are placed in a sealed plastic bag labeled with the name of the person to receive the product and the name and location of the receiving facility.

In addition to SoftBank documentation described above, product distribution is documented for all products on a Clinical Product Distribution log, with the following elements recorded:

- Initials of CPS person releasing product to clinical care staff, courier or shipper
- Name of clinical care staff, courier, or shipper picking up product
- Date and time of product release from CPS to clinical care staff, courier, or shipper
- Name and location or address of person to receive product
- Product name
- Product identifier(s)
- Recipient identifier(s)

XIII. Final Product Preparation, Issue, and Administration

A. Final Product Preparation

1. Medical Order for Product Infusion/Administration

Final preparation of the product is performed by CPS according to protocol-specific requirements (see Section VIII, Manufacturing Systems and Process Controls). The medical order for product infusion/administration, which contains the names and identifiers of the intended product recipient and a list and details of patient-specific products to be administered on a given date according to protocol-specific requirements, is generated in CPS. The medical order is reviewed and signed by the protocol PI or designee, and returned to CPS before final product preparation. Any special instructions for final product preparation are part of this medical order.

2. Methods for Final Product Preparation

Final preparation of the product may include thawing, transfer to another container, volume reduction, dilution, resuspension in another infusion solution, or gamma irradiation. These steps are considered an extension of the manufacturing process and are performed by trained technical staff using validated methods. Special considerations for cryopreserved, fresh, and non-cryopreserved cultured products are as follows:

- Cryopreserved products are removed from LN2 storage, thawed and issued in accordance with DTM-SOP-5072 (Cryopreservation of Human Cells Using Pentastarch and Dimethyl Sulfoxide) and DTM-SOP-5014 (Disposition of Cryopreserved HPC Components in CPS Storage). These procedures include clerical checks at the time the product is removed from storage, at the time of preparation of the dilution/infusion solution, and at the time of transport to the patient care unit for infusion. Patient identification during the issuing process is confirmed by a series of clerical checks performed by trained, authorized personnel.

- Fresh products (e.g., PBSC and lymphocytes), which typically are minimally manipulated, may be issued on the day of collection or up to 24 hours from the time of collection. These products are held at monitored room temperature from the time of lot release until the time of issue.

- For non-cryopreserved cultured products, on the day of administration, the cells are harvested and the final cellular product goes through lot release procedures and is packaged in its final container for pick-up and transport to the patient care unit by designated, authorized personnel.

B. Product Issue and Other Disposition

Product issue, which is defined as on-site distribution, follows the policies and procedures for product distribution, summarized in Section XII (Product Receipt and Distribution). Documentation of issue or other disposition is done at the time of issue or other disposition and includes:

- Computer entry in the Soft Bank computer system indicating issue or other disposition of the product, as an addendum to the product entry that had been made immediately after its manufacture
- Written notation on both the CPS patient product summary record and the CPS product record indicating the final product disposition

Products are only issued to patients for IRB-approved clinical protocols for which the products were manufactured.

Disposition of the product other than issue for use by the patient for which the product was manufactured includes discard or transfer for research use. These alternative dispositions are carried out only in accordance with procedures defined in IRB-approved protocols and with CPS policies requiring the explicit approval of both the protocol PI and the CPS Medical Director.

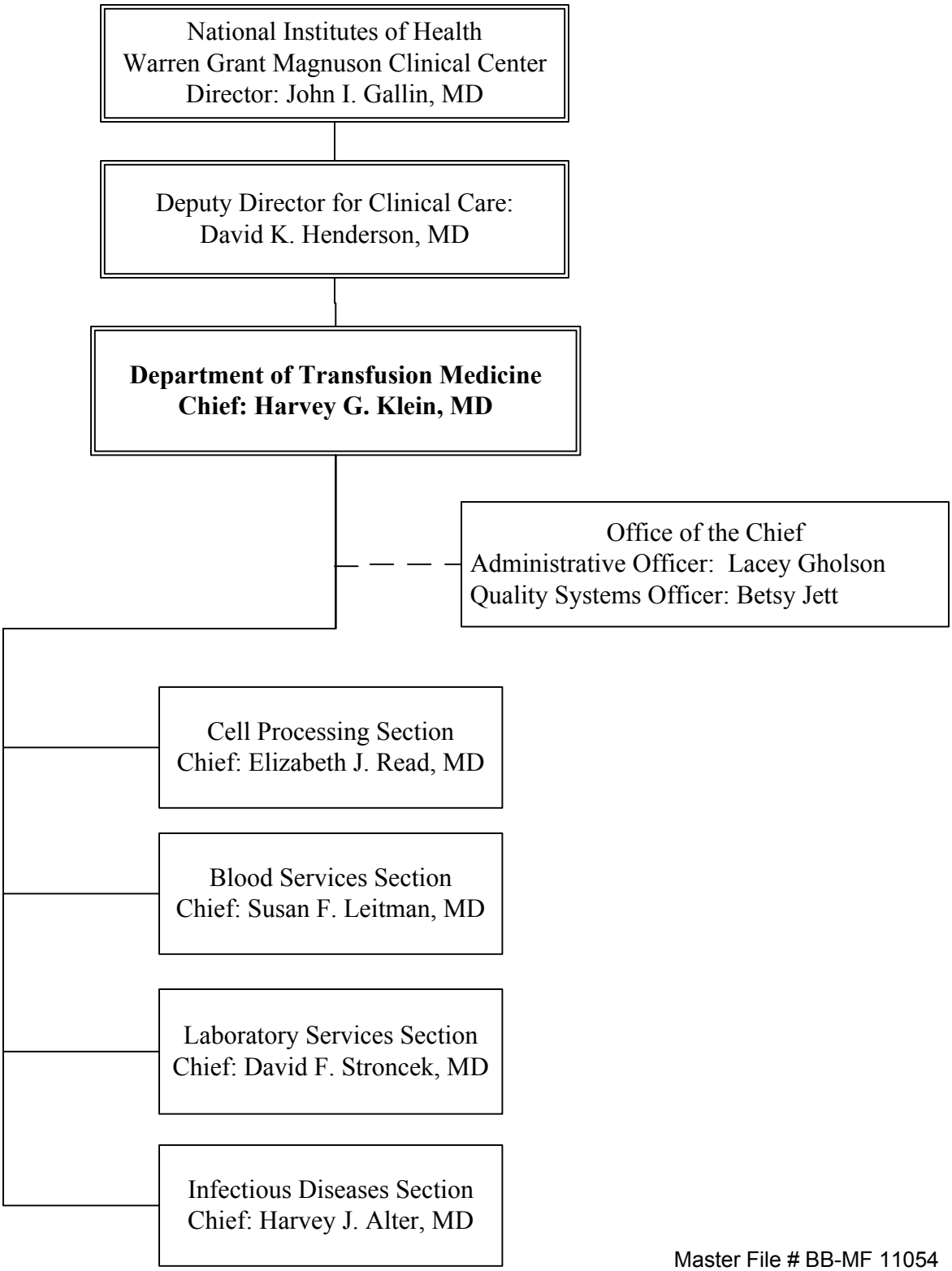
C. Product Administration

On NIH CC patient care units, cellular therapy products are administered in accordance with a CC Department of Nursing SOP (Infusion of Products for Cellular Therapy) developed and implemented by the CC Department of Nursing, after review and concurrence by the CC DTM. This SOP includes:

- Medical guidelines for use of patient premedication
- Requirements for verification of the patient's identity and a match of patient identifiers with product identifiers prior to product administration by two trained persons
- Requirements for monitoring of the patient during and after product administration
- Requirements for documentation of the details of product administration in the medical record
- Requirements for medical management and documentation of adverse events in the medical record

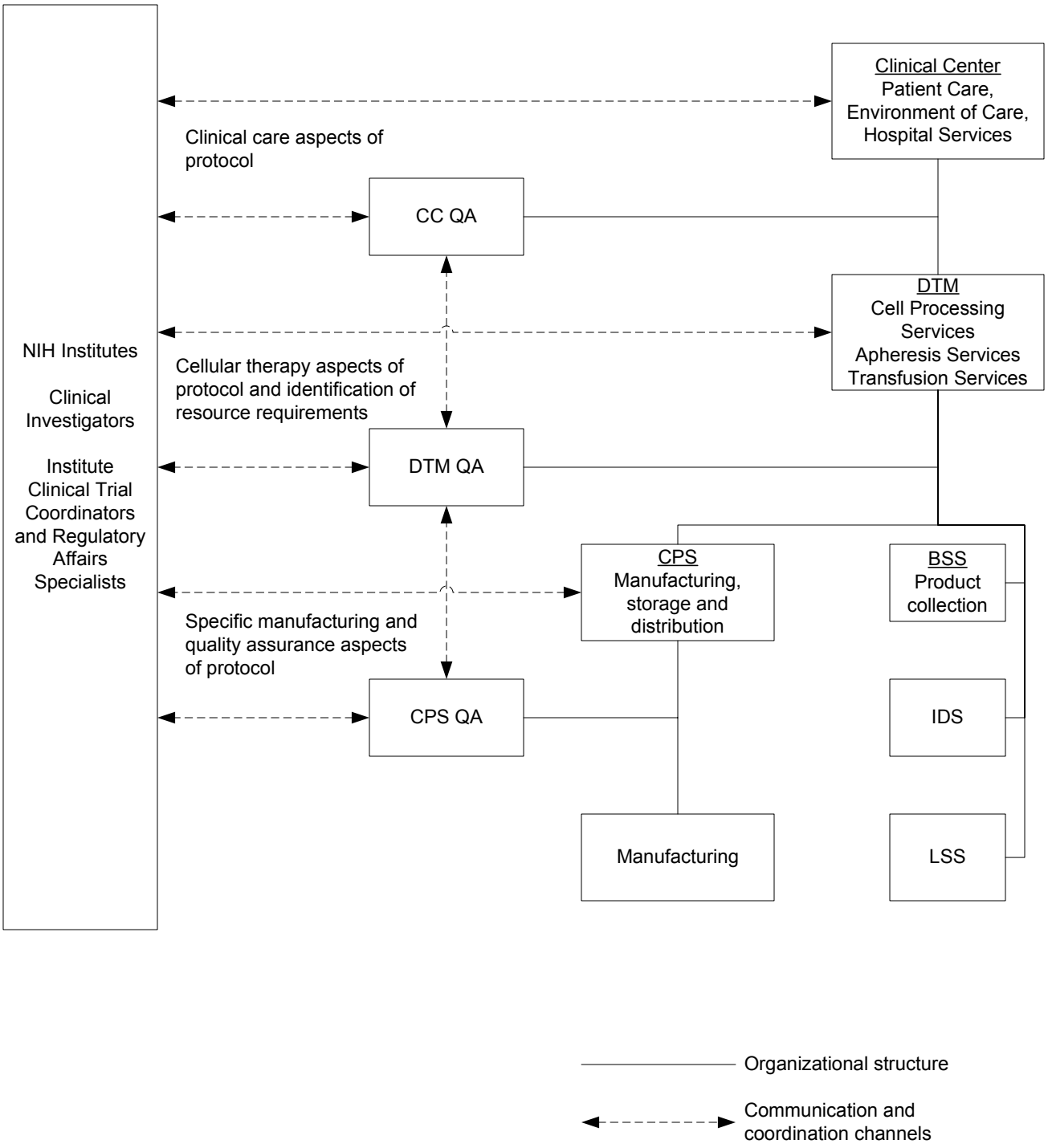
XIV. Attachments

Organizational Chart



Cell Processing Section
Department of Transfusion Medicine
NIH Clinical Center

Relationship Between Institute Investigators and CC Regarding Protocol Design, Implementation and Quality Assurance



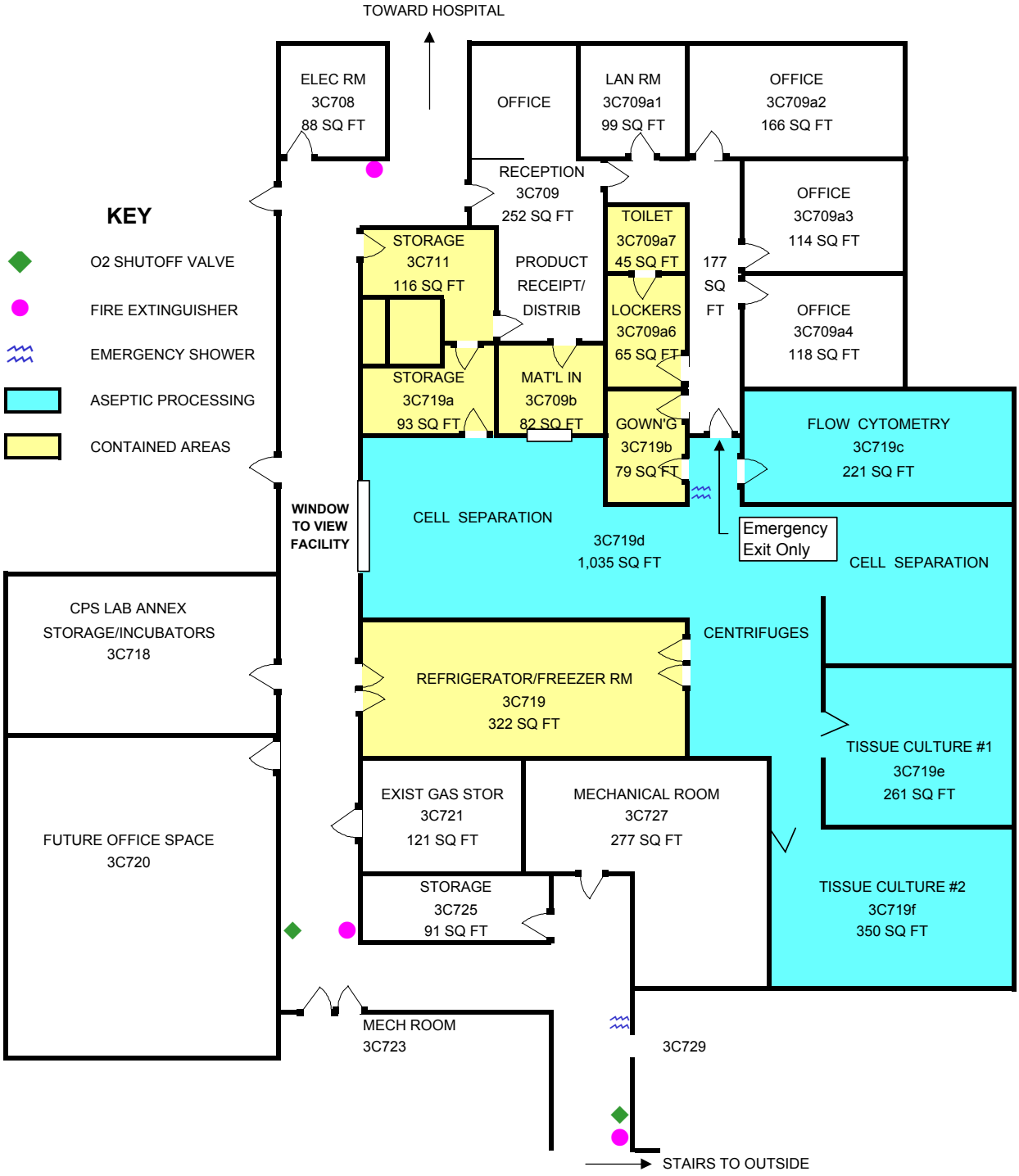
QUALITY CONTROL SCHEDULE

EQUIPMENT	QC PROCEDURE	FREQUENCY OF PERFORMANCE					
		DAILY	WEEKLY	MONTHLY	QRT'LY	YEARLY	ALSO
Refrigerators/Freezers	Record Temperature	X					
	Clean/Discard exp. reagents			X			
Liquid N_2 freezers	Record N_2 level	X					
Biological cabinets	Clean			X			
	Clean air filters					X	
	Calibrate airflow					X	
Water Baths	Record temperature	X					
	Clean			X			
Incubators	Record temperature	X					
	Record CO_2 level	X					
	Check water level		X				
	Clean/Autoclave				X		
Centrifuges	Clean			X			
	Preventive maintenance					X	
Sterile Connectors	Record lot #	X					
	Validate weld	On use					
Trypan Blue	Replace			X			
Cell Dyn	Run Controls	X					
	Calibrate					X	As needed
	Preventive maintenance			X	X		As needed

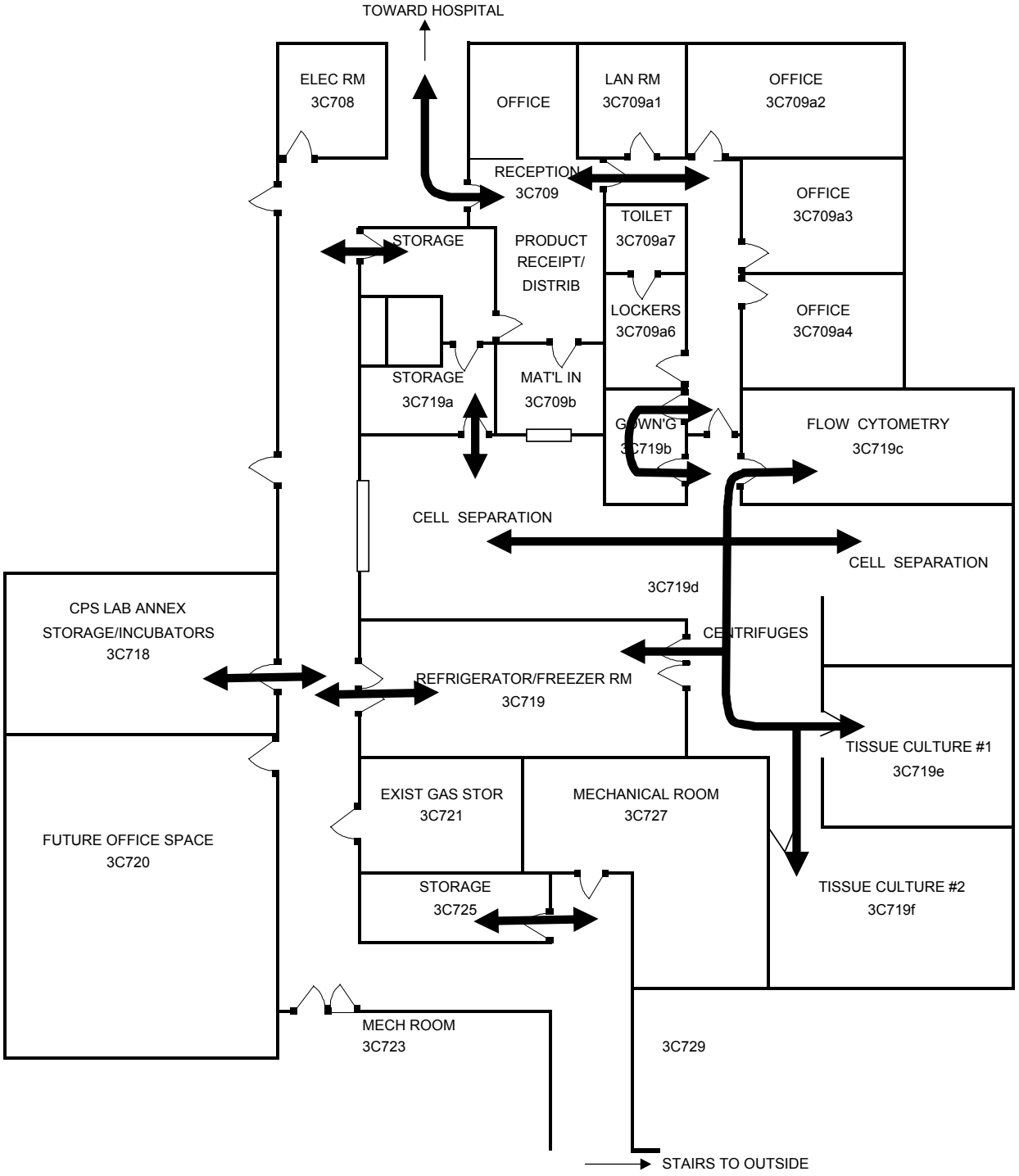
QUALITY CONTROL SCHEDULE

EQUIPMENT	QC PROCEDURE	FREQUENCY OF PERFORMANCE					
		DAILY	WEEKLY	MONTHLY	QRT'LY	YEARLY	ALSO
	Analyze controls data				X		
Sinks	Clean		X				
Sharps containers	Check for fullness		X				
Timers/Stopwatch	Calibrate					X	On receipt
Thermometers	Calibrate					X	On receipt
Pipettors	Calibrate					X (2)	On receipt
Scales/Balances	Check accuracy			X			
	Calibrate				X		On receipt
CS3000	Systems check			X			
	Preventive maintenance					X (2)	
Eyewashers	Flush / Clean		X				
Epics Flow cytometers	Run Flow Check / Flow Set	X					
	Run Cleaning Panels	X					
	Run daily instrument checks	X					
	Clean probes / ext. surfaces		X				
	Run ADC		X				
	Clean air filters / tanks				X		
	Preventive Maintenance					X	As needed
Isolex 300i	Preventive Maintenance					X (2)	As needed

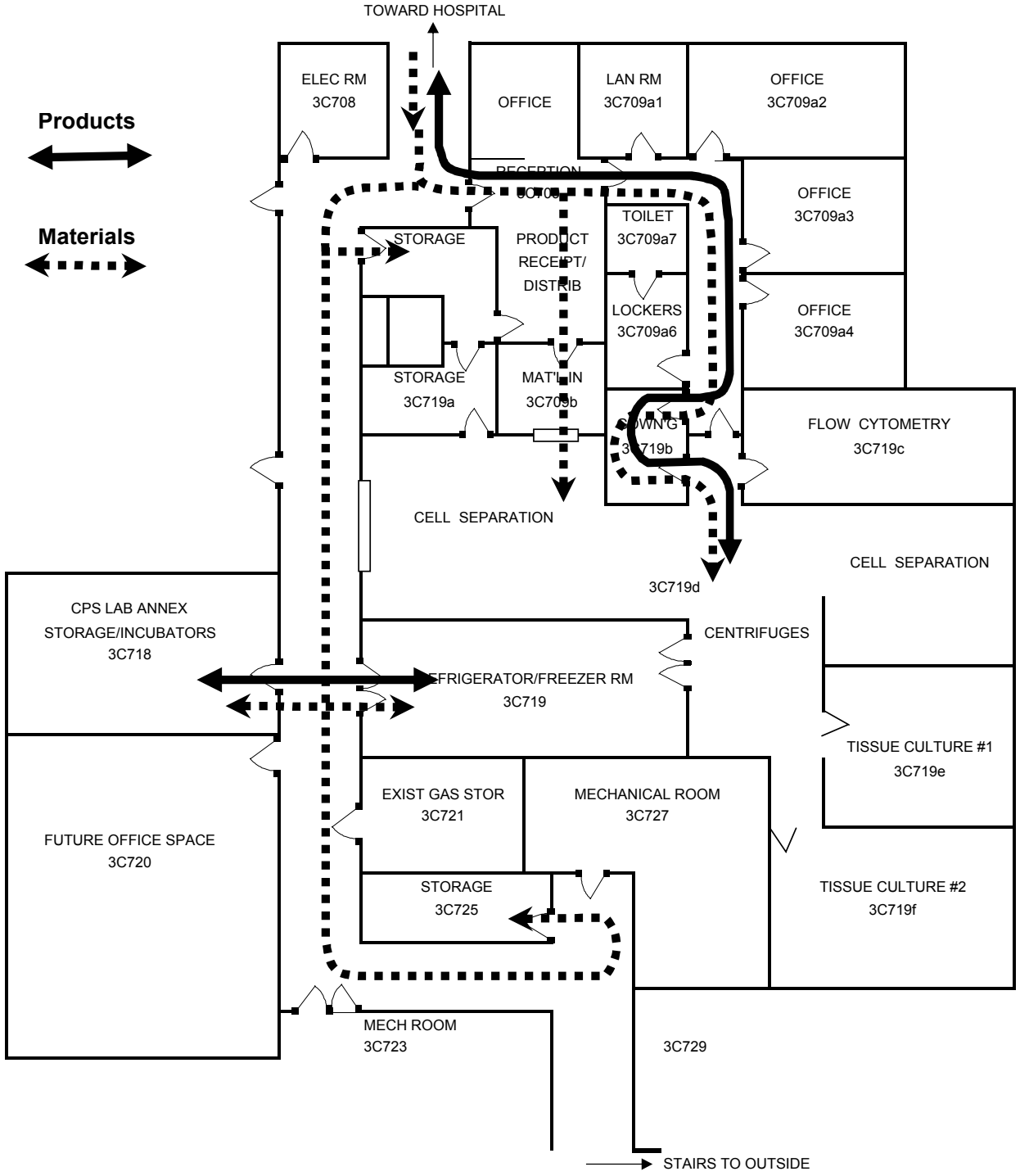
CPS Facility Floorplan



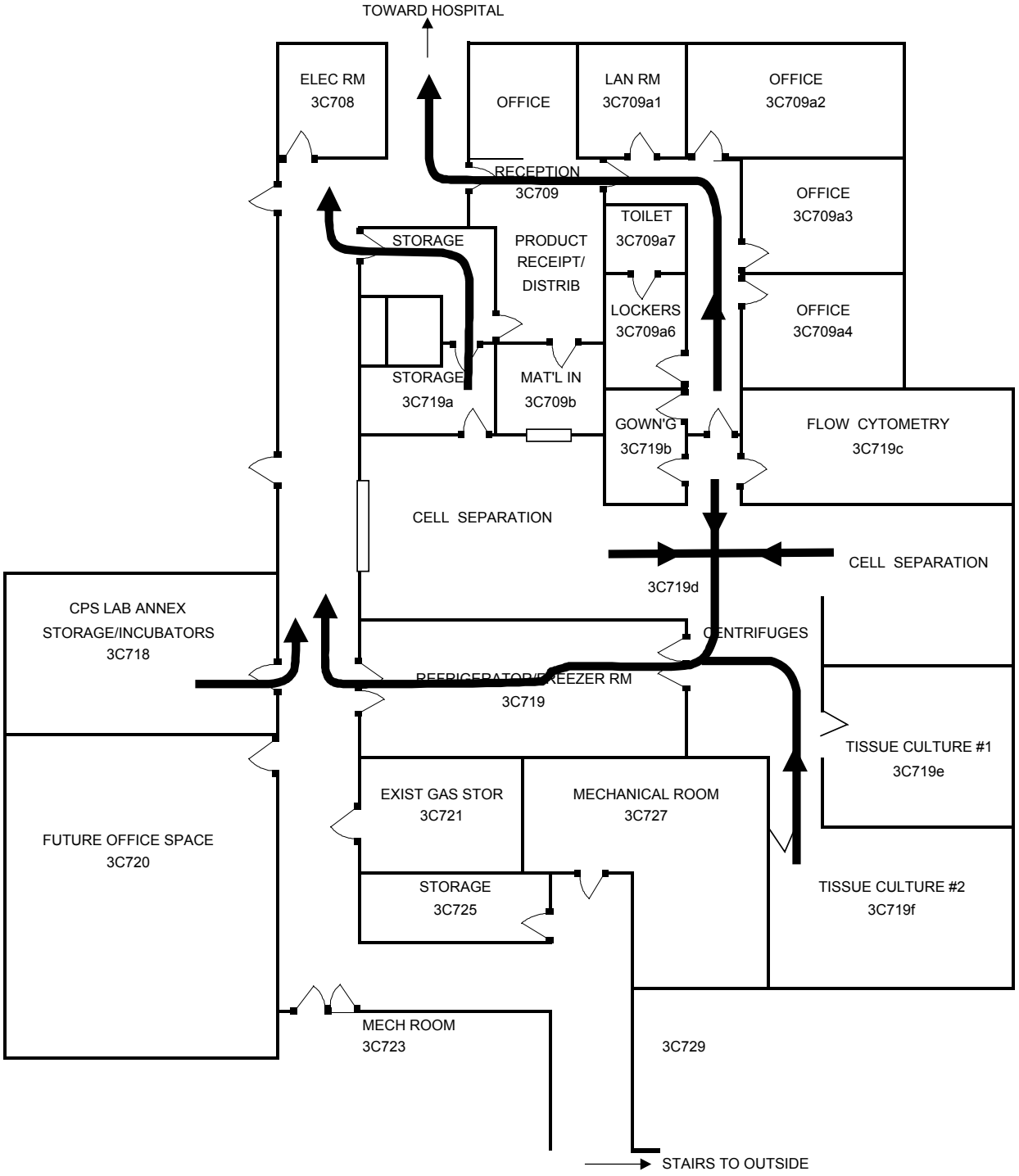
Personnel Flow Within CPS Facility



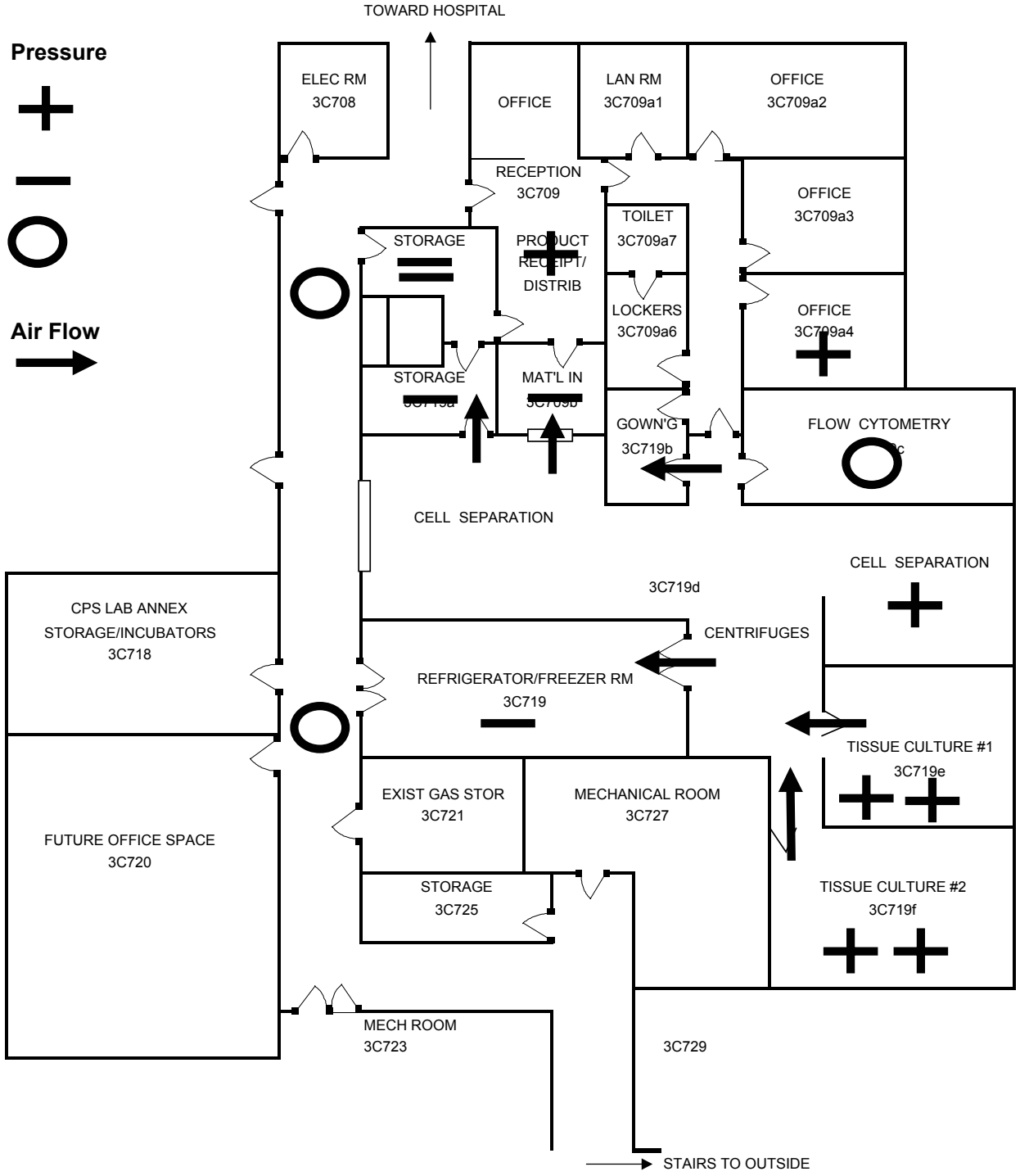
Product and Materials Flow Within CPS Facility



Waste Flow Within CPS Facility



Air Pressure Differentials and Flow Within CPS Facility



Attachment 9-A	**Donor Selection & Eligibility for Living Autologous Donors**
Selection	Each donor is a patient who meets selection criteria outlined in an IRB-approved clinical protocol. Protocol PI evaluates donor-patient's eligibility for protocol according to selection (inclusion/exclusion) criteria including age, disease, disease status or stage, organ function criteria, absence of certain infections, and performance status. Each donor-patient is evaluated by protocol PI and/or DTM staff for ability to tolerate cell collection procedure and any related line placement and anesthesia, if applicable.
Screening for Transmissible Disease	Donor health history screening: Apheresis donors: questionnaire compliant with FDA and AABB requirements for blood donors is administered by DTM apheresis staff (1) at time of venous assessment and (2) on day of collection. Bone marrow donors: general health history screening by PI Donor blood testing is performed (1) within 30 days prior to collection (prescreen) and (2) on day of collection for: HIV-1,2, by anti-HIV (EIA) and HIV-1 RNA (NAT) HBV, by HBsAg (EIA) and anti-HBc (EIA) HCV, by anti-HCV (EIA) and HCV RNA (NAT) HTLV-I/II, by anti-HTLV-I/II (EIA) Treponema pallidum (RPR by latex agglutination)
Transmissible Disease Acceptance Criteria	Abnormal results are not cause for ineligibility of donor or rejection of product unless listed in protocol exclusion criteria.
Documentation of Abnormal or Incomplete Results on Transmissible Disease Screening	Abnormal results on donor health history questionnaire are documented on apheresis donor record at both times of questionnaire administration. Abnormal or incomplete results on donor blood testing are documented on prescreen checklist and on product record. For products with abnormal or incomplete results: - acceptance for storage requires approval of DTM physician - release for patient use requires approval of DTM physician and protocol PI
Notification for Abnormal Donor Screening Results	Abnormal results on donor health history questionnaire are communicated, if relevant in the autologous setting, to the PI or designee by DTM physician. Abnormal results on donor blood testing are communicated, if relevant in the autologous setting, to the PI or designee by DTM physician.

Attachment 9-B	**Donor Selection & Eligibility for Living Allogeneic, Family Related Donors of Products Other than Cord Blood**
Selection	Each donor is a first degree family member who meets selection criteria outlined in an IRB-approved clinical protocol. Protocol PI evaluates donor's eligibility for protocol according to selection (inclusion/exclusion) criteria including age, HLA matching with recipient, organ function criteria, and absence of certain infections. Each donor is evaluated by protocol PI and/or DTM staff for ability to tolerate cell collection procedure and any related line placement and anesthesia, if applicable.
Screening for Transmissible Disease	Donor health history screening: Apheresis donors: questionnaire compliant with FDA and AABB requirements for blood donors is administered by DTM apheresis staff (1) at time of venous assessment and (2) on day of collection Bone marrow donors: health history screening performed by PI Donor blood testing is performed (1) within 30 days prior to collection (prescreen) and (2) on day of collection for: HIV-1,2, by anti-HIV (EIA) and HIV-1 RNA (NAT) HBV, by HBsAg (EIA) and anti-HBc (EIA) HCV, by anti-HCV (EIA) and HCV RNA (NAT) HTLV-I/II, by anti-HTLV-I/II (EIA) Treponema pallidum (RPR by latex agglutination) CMV testing of donor blood by ELISA for anti-CMV IgG + IgM, by DLM Immunology Lab, prior to final selection of donor, with result recorded in donor medical record and in transplant chart
Transmissible Disease Acceptance Criteria	Abnormal results are not cause for ineligibility of donor or rejection of product unless listed in protocol exclusion criteria. Decisions about acceptance of CMV positive donor and management of recipient of CMV positive product are detailed in clinical protocol.
Documentation of Abnormal or Incomplete Results on Transmissible Disease Screening	Abnormal results on donor health history questionnaire are documented on apheresis donor record at both times of questionnaire administration. Abnormal or incomplete results on donor blood testing are documented on prescreen checklist and on product record. For products with abnormal or incomplete results: - acceptance for storage requires approval of DTM physician - release for patient use requires approval of DTM physician and protocol PI
Notification for Abnormal Donor Screening Results	Abnormal results on donor health history are communicated to the PI or designee by DTM physician. Abnormal results on donor blood testing are communicated to the PI or designee by DTM physician.

Attachment 9-C	**Donor Selection & Eligibility for Cord Blood Donors (Autologous or Allogeneic Family-Related)**
Selection	Each donor is an infant with risk for sickle trait or disease, other hemoglobinopathy, or other genetic disease Eligibility of mother/infant is assessed according to selection (inclusion/exclusion) criteria outlined in IRB-approved protocol. Mother must be negative for HIV, HBV, and HCV.
Screening for Transmissible Disease	Donor health history screening is performed on mother prior to delivery of infant and cord blood collection, using donor health history questionnaire compliant with FDA and AABB requirements. Donor blood testing is performed on cord blood on day of collection for: HIV-1,2, by anti-HIV (EIA) and HIV-1 RNA (NAT) HBV, by HBsAg (EIA) and anti-HBc (EIA) HCV, by anti-HCV (EIA) and HCV RNA (NAT) HTLV-I/II, by anti-HTLV-I/II (EIA) Treponema pallidum (RPR by latex agglutination) CMV, by CMV Ag (PCR)
Transmissible Disease Acceptance Criteria	Criteria to be established when clinical protocol involving use of cord blood products is established.
Documentation of Abnormal or Incomplete Results on Transmissible Disease Screening	Abnormal results on donor health history are documented on donor health history record. Abnormal or incomplete results on donor blood testing are documented on product record. For products with abnormal or incomplete results: - acceptance for storage requires approval of DTM physician - release for patient use, if and when IRB-approved clinical protocol is established, will require approval of DTM physician and PI
Notification for Abnormal Donor Screening Results	Abnormal results on cord blood testing are communicated to mother by DTM nurse coordinator. Policy for notification of abnormal results on donor health history or cord blood testing to PI to be established when clinical protocol for use of cord blood is established.

Attachment 9-D	**Donor Selection & Eligibility for Cadaveric Donors**
Selection	Potential donors are identified in external health care facility. The organ procurement organization (OPO) is responsible for initial selection procedures. After initial selection by the OPO, the cadaveric donor is selected according to criteria written in NIH IRB-approved protocol(s).
Screening for Transmissible Disease	Donor medical history questionnaire compliant with UNOS standards for organ and tissue donors is administered by OPO staff at time of obtaining consent from the next of kin. Donor blood is tested by a CLIA-certified lab, under contract with the OPO, for HIV-1,2 by anti-HIV-1,2 (Abbott EIA) HBV, by HBsAg, anti-HBs, and anti-HBc (all Abbott EIA) HCV by anti-HCV (Abbott EIA) HTLV-I/II by anti-HTLV-I/II (Abbott EIA) Treponema pallidum by RPR Other tests include CMV (anti-CMV latex agglutination) and Toxoplasmosis (Ab testing)
Transmissible Disease Acceptance Criteria	All donor screening results are reviewed by PI before acceptance of organ or tissue. If HIV, HBV, or HCV are positive, organs or tissues are not used, unless otherwise specified in protocol. If anti-CMV antibody is positive, CMV prophylaxis is administered to recipient if specified in protocol.
Documentation of Abnormal or Incomplete Results on Transmissible Disease Screening	Copies of donor risk assessment questionnaire and documentation of the results of the transmissible disease testing are sent and delivered at the same time as the organ or tissue, and are kept in product record. Certificate of analysis includes verification that screening results were reviewed by PI, and that donor met criteria specified in protocol.
Notification for Abnormal Donor Screening Results	Notification is the responsibility of the OPO.

Attachment 10. Cell Separation & Selection Procedures

Procedure	Purpose	Starting cellular material	Method and/or instrument
Plasma removal	Reduce volume of incompatible plasma in cases of minor ABO incompatibility or red cell alloantibody	Mononuclear cells PBSC Lymphocytes (DLI)	Centrifugation in Sorval floor model centrifuge, followed by expression of plasma into another bag
Red cell sedimentation	Reduce RBC content of cellular products in cases of major ABO incompatibility	PBSC Lymphocytes (DLI)	Hydroxyethyl starch (Hespan) and gravity sedimentation in blood bag, followed by expression of RBC-reduced product into another bag
Counterflow centrifugal elutriation	Separate mononuclear cell products into monocyte and lymphocyte fractions for further manufacture or final products	Mononuclear cells	JE-5.0 elutriator and rotor (Beckman)
Closed system elutriation	Separate mononuclear cell products into monocyte and lymphocyte fractions for further manufacture or final products.	Mononuclear cells	Spectra Cell elutriation system (Gambro BCT) Method in development; will require Device MF crossreference in IND application
Manual buffy coat	Concentrate nucleated cells and reduce the volume and red cell content of harvested bone marrow or PBSC	Bone marrow PBSC	Centrifugation in Sorval floor model centrifuge, followed by expression of plasma and buffy coat WBCs into another bag
Inverted spin	To remove fat from harvested bone marrow	Bone marrow	Centrifugation in Sorval floor model centrifuge, followed by gravity drainage of cells and plasma
Manual density gradient	Purify mononuclear cells (by removing RBCs and granulocytes) from bone marrow or mononuclear cell products	Bone marrow Mononuclear cells	Centrifugation of product after layering on Lymphocyte separation medium (Ficoll-hypaque 1.077) in sterile conical tubes, followed by pipetting of MNC layer from gradient

Procedure	Purpose	Starting cellular material	Method and/or instrument
Automated (closed system) density gradient	Purify mononuclear cells (by removing RBCs and granulocytes) from bone marrow or mononuclear cell products	Bone marrow Mononuclear cells	Automated procedure on Fenwal CS3000 using Lymphocyte separation medium (Ficoll-hypaque 1.077) and plastic blood bags
Automated cell harvest	Concentrate cells from large volume tissue culture	Cultured cells (usually mononuclear cells) in large volume	Automated procedure using Fenwal CS3000 or Cobe 2991 blood cell processor
Automated closed system immunomagnetic cell selection (Isolex)	(1) Purify CD34+ cells and/or to reduce T-cell content of HPC products or (2) Enrich for cell populations by negative selection (e.g., CD3/CD19 depletion to enrich for CD14 monocytes)	PBSC Bone marrow Cord blood Mononuclear cells	Isolex 300i Magnetic cell separator (Baxter), disposable set, specific antibodies, and SAM beads IND/IDE with crossreference to Device MF required for some applications
Semi-automated closed system immunomagnetic cell selection (CliniMacs)	(1) Purify CD34+ or AC133+ cells and/or to reduce the T cell content of HPC products or (2) Positive selection of other cell populations	PBSC Bone marrow Cord blood Mononuclear cells Cultured lymphs	CliniMACS magnetic cell separator (Miltenyi), disposable set, specific antibodies conjugated to paramagnetic microparticles IND/IDE with crossreference to Device MF required for all applications
Semi-automated closed system immunomagnetic cell selection (MaxSep)	Deplete cell populations from a cellular product (e.g., CD8 depletion to enrich CD4 cells)	Mononuclear cells Cultured lymphs	Use of Maxsep selection device (Baxter) to magnetically remove cells specific antibodies, SAM beads

Attachment 11-A. Donor Transmissible Disease Testing	
Assay category	Safety Release assay
Agents	See Section VII (Donor Selection and Eligibility) and Attachment 9 A-D
Sample type(s)	Donor blood, prescreen Donor blood, day of collection Cord blood, day of collection
Testing lab & accreditation	CC DTM: AABB, CLIA (ID# 21D0662289) CC DLM: CAP, CLIA (ID# 21D0665373)
Purpose of assay	Screen donors for presence or risk of transmissible disease
Method(s)	See Section VII (Donor Selection and Eligibility) and Attachment 9 A-D
Assay limits	Negative or nonreactive
Confirmatory or supplemental assay(s)	Confirmatory or supplemental assays are performed depending on screening assay results and agent/marker
Action plan for abnormal result	See Section VII (Donor Selection and Eligibility) and Attachment 9 A-D

Attachment 11-B. Sterility Testing (Bacterial and Fungal Cultures)	
Assay category	Safety In-process and release assay
Sample type(s)	Product, in-process Product, final
Testing lab & accreditation	CC DLM: CAP, CLIA (ID# 21D0665373)
Purpose of assay	Detect bacteria or fungi in product
Method(s)	CFR/USP compliant method using Thioglycollate and Tryptic Soy Broth media. Readings performed 2x/week Automated blood culture method: BacT/ALERT FA (aerobic) and FN (anaerobic) media (BioMerieux, Durham, NC). System monitors for evidence of CO2 production, as indicator of microbial growth, q 10 min Both methods performed in parallel, with 14-day incubation
Assay limits	No growth
Confirmatory or supplemental assays	Gram stain, growth on solid media, biochemical profile, sensitivity testing, and genotyping, as necessary
Action plan for abnormal result	Positive cultures are communicated by telephone to DTM physician immediately. For product not yet infused, positive culture communicated by DTM physician to PI as soon as possible and always before scheduled infusion. PI determines whether product will be administered to the patient. For product already infused, positive culture is communicated by DTM physician to PI immediately, so that PI can make contact with patient to determine further medical interventions. PI is responsible for notification of the IRB and FDA, in accordance with requirements of the protocol and IND.

Attachment 11-C. Mycoplasma PCR Testing	
Assay category	Safety Release assay, but results may be pending at time of release
Sample type(s)	Product, final
Testing lab & accreditation	CC-DLM: CAP, CLIA (ID# 21D0665373)
Purpose of assay	Detect the presence of Mycoplasma/acholeplasma in product
Method(s)	Mycoplasma PCR ELISA (Roche Diagnostics, Mannheim, Germany) Photometric enzyme immunoassay for the detection of PCR-amplified DNA of Mycoplasma/acholeplasma in cell culture Detects all (6) species encountered in cell cultures
Assay limits	Negative
Confirmatory or supplemental assay(s)	Assay currently being done in parallel with Mycoplasma culture
Action plan for abnormal result	For product not yet infused, positive result communicated by DTM physician to PI as soon as possible and always before scheduled infusion. PI determines whether product will be administered to the patient. For product already infused, positive result is communicated by DTM physician to PI immediately, so that PI can make contact with patient to determine further medical interventions. PI is responsible for notification of the IRB and FDA, in accordance with requirements of the protocol and IND.

Attachment 11-D. Mycoplasma Culture	
Assay category	Safety Release assay, but results may be pending at time of release
Sample type(s)	Product, final
Testing lab	Mycoplasma Laboratory, NCI-FCRDC (Frederick, MD)
Purpose of assay	Detect the presence of Mycoplasma in product
Method(s)	Standard axenic culture using CMRL axenic medium rather than indicator cells for the readout, and for the isolation of all strains of Mycoplasma hyporhinis. Method demonstrated to be comparable in sensitivity and specificity to the method described in the 1993 FDA Points to Consider Document. Reference: Del Giudice, RAM. CMRL, A new axenic medium to replace indicator cell cultures for the isolation of all strains of Mycoplasma hyporhinis. In Vitro Cell Div Biol-Animal 1998;34:88-89.
Assay limits	No growth
Confirmatory or supplemental assay(s)	Assay currently being done in parallel with Mycoplasma PCR
Action plan for abnormal result	CPS will notify the PI immediately for a decision about whether product will be administered. For product not yet infused, positive culture result communicated by DTM physician to PI as soon as possible and always before scheduled infusion. PI determines whether product will be administered to the patient. For product already infused, positive culture result is communicated by DTM physician to PI immediately, so that PI can make contact with patient to determine further medical interventions. PI is responsible for notification of the IRB and FDA, in accordance with requirements of the protocol and IND.

Attachment 11-E. Endotoxin Testing	
Assay category	Safety Release assay
Sample type(s)	Product, final
Testing lab & accreditation	CC DTM: AABB, CLIA (ID# 21D0665373)
Purpose of assay	Detect presence of bacterial endotoxin in product
Method(s)	BioWhittaker LAL Pyrogent-5000 assay, performed according to manufacturer's instructions and DTM-SOP-5067 (Determination of Endotoxin Level Using the Pyrogent 5000 Turbidometric Limulus Amebocyte Lysate (LAL) Kinetic Assay). Assay description: - designed as in vitro end-product endotoxin test - quantitative, kinetic assay - readout based on detection of increased turbidity (optical density) after endotoxin catalyzes LAL proenzyme to coagulase, which hydrolyzes coagulogen and forms gelatinous clot.
Assay limits	< 5 EU per ml
Confirmatory or supplemental assay(s)	Low level results, even if below limits, may be repeated and evaluated for presence of interfering substances
Action plan for abnormal result	Results not meeting limits are called immediately to the PI. The product is not released, and investigation of source of endotoxin is initiated. PI responsible for notification of IRB and FDA, in accordance with requirements of the protocol and IND.

Attachment 11-F. Automated Cell Counting	
Assay category	Dose, Identity, Purity In-process or release assay
Sample type(s)	Donor blood, pre-apheresis Donor blood, post-apheresis Cellular source material Product, in-process Product, final
Testing lab & accreditation	CC DTM: AABB, CLIA (ID# 21D0665373) Proficiency testing: CAP survey
Purpose of assay	Quantitate cells and other hematologic parameters in source material and products
Method(s)	CellDyn 4000 automated cell counter (Abbott), using DTM-SOP-0082 (Operation of the CellDyn 4000) Uses laser optics and electrical impedance to analyze cells Measures WBC, WBC diff, RBC count, platelet count, Hb Calculates other hematologic values (MCV, RDW, etc.)
Assay limits	Product cellular content is defined in protocol/IND
Confirmatory or supplemental assay(s)	None
Action plan for abnormal result	PI is notified if product cellular content values do not meet target or minimum doses as defined in protocol/IND

Attachment 11-G. Trypan Blue Viability Testing	
Assay category	Dose, Potency In-process or release assay
Sample type(s)	Product, final
Testing lab & accreditation	CC DTM: AABB, CLIA (ID# 21D0662289)
Purpose of assay	Quantitate viability of cells in entire product
Method(s)	Staining with trypan blue, then manual cell count by hemocytometer method. Cells excluding trypan blue are viable.
Assay limits	Defined in protocol/IND
Confirmatory or supplemental assay(s)	Flow cytometric viability assay (7AAD), as needed or as described in specific protocol
Action plan for abnormal result	PI is notified if viability limits defined in protocol/IND are not met

Attachment 11-H. Flow Cytometric 7-AAD Viability Testing	
Assay category	Dose, Potency In-process or release assay
Sample type(s)	Product, in-process Product, final
Testing lab & accreditation	CC DTM: AABB, CLIA (ID# 21D0662289)
Purpose of assay	Quantitate viability of cells in entire product and/or of a cell population within the product. Also used in combination with phenotypic marker(s) to quantitate viable cells (as opposed to all cells) of a given phenotype by flow cytometry.
Method(s)	Staining with 7AAD (which binds to DNA), then flow cytometric quantitation and characterization of cell populations. Cells excluding 7-AAD are viable.
Assay limits	Defined in protocol/IND
Confirmatory or supplemental assay(s)	Trypan blue viability, as needed or as described in specific protocol
Action plan for abnormal result	PI is notified if viability limits defined in protocol/IND are not met

Attachment 11-I. Flow Cytometric Phenotyping of Cells	
Assay category	Dose, Identity, Purity In-process or release assay
Sample type(s)	Product, in-process Product, final
Testing lab & accreditation	CC DTM: AABB, CLIA (ID# 21D0662289) Proficiency testing: CAP surveys
Purpose of assay	Characterize cell populations by cell surface marker expression, and (in combination with cell counting) quantitate cells of a certain cell population in product
Method(s)	Beckman Coulter EPICS XL flow cytometer Sample preparation according to DTM-SOP-5092 (Sample Preparation for Flow Cytometric Analysis) Fluorochromes used (4 channels): FITC, PE, ECD (PE+Texas red), and PC5 (PE+Cy5) 7-AAD may be used as 4th color
Assay limits	Defined in protocol/IND
Confirmatory or supplemental assay(s)	None
Action plan for abnormal result	PI is notified if assay limits defined in protocol/IND are not met

Attachment 11-J. Hematopoietic Colony Assays	
Assay category	Potency Not a release assay but reviewed retrospectively
Sample type(s)	Product, final (all products) Product, post-thaw (2 per month for QC)
Testing lab & accreditation	CC DTM: AABB, CLIA (ID# 21D0662289) Proficiency testing: Stem Cell Technologies (Vancouver, Canada) tests 1,2,3
Purpose of assay	Quantitate product content of hematopoietic progenitor cells with clonigenic capacity
Method(s)	Standardized method plating defined number of nucleated cells on semisolid methylcellulose medium + growth factors, followed by quantitation of CFU-GM, BFU-E, and CFU-GEMM colonies after 14 days of incubation.
Assay limits	Growth of colonies
Confirmatory or supplemental assay(s)	None
Action plan for abnormal result	No actions taken for abnormal result unless investigation is initiated in case of engraftment failure

Attachment 11-K. Testing for ABO Group, Rh Type, and Unexpected RBC Antibodies	
Assay category	Identity In-process or release assay
Sample type(s)	Donor blood, pre-screen Donor blood, day of collection
Testing lab & accreditation	CC DTM: AABB, CLIA (ID# 21D0665373)
Lab accreditation	AABB accreditation
Method(s)	MTS Gel Card System (Micro Typing Systems, Pompano Beach, FL)
Assay limits	Defined by protocol/IND: in general, results not used to accept/reject product, but to determine need for additional manipulations (see Action plan, below)
Confirmatory or supplemental assay(s)	None
Action plan for abnormal result	Defined by protocol/IND For products containing significant numbers of RBCs: If major ABO incompatibility between donor & recipient is detected by pre-screen testing, RBC sedimentation procedure is performed to minimize acute hemolytic transfusion reaction after product infusion. If minor ABO incompatibility between donor & recipient or donor alloantibody is detected by pre-screen testing, plasma depletion procedure is performed to minimize acute hemolytic transfusion reaction after product infusion.

Attachment 11-L. HLA Antibody Screening	
Assay category	Purity, Safety Release assay
Sample type(s)	Donor serum
Testing lab & accreditation	CC DTM: ASHI-05-2-MD-09-01 (deemed status for CLIA (ID# 21D0662289)
Purpose of assay	To verify the absence of HLA antibodies in donors of allogeneic plasma collected and manufactured into heat-inactivated plasma or serum (used as an ancillary product during cell culture)
Method(s)	Lambda Antigen Tray (LAT-140) for ELISA Photometric enzyme assay that detects IgG antibodies in donor serum directed against HLA class I (A, B) antigens
Assay limits	Negative (≤ 10% panel reactive antibody (PRA))
Confirmatory or supplemental assay(s)	None
Action plan for abnormal result	If donor HLA Ab screen is positive, the plasma or serum lot from that donation is rejected.